

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
31 March 2005 (31.03.2005)

PCT

(10) International Publication Number  
**WO 2005/028679 A2**

- (51) International Patent Classification<sup>7</sup>: **C12Q 1/68**
- (21) International Application Number:  
**PCT/US2004/029602**
- (22) International Filing Date:  
**13 September 2004 (13.09.2004)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:  
**10/661,094 12 September 2003 (12.09.2003) US**
- (71) Applicant (for all designated States except US): **UNIVERSITY OF IOWA RESEARCH FOUNDATION [US/US];**  
Technology Innovation Center, Suite #214, 100 Oakdale  
Campus, Iowa City, IA 52242 (US).
- (71) Applicant and  
(72) Inventor: **DODGSON, Kirsty, Jane [GB/US];** 124 Grove  
Street, Iowa City, IA 52246 (US).
- (74) Agents: **STEFFEY, Charles, E. et al.;** Schwegman, Lund-  
berg, Woessner & Kluth, P.O. Box 2938, Minneapolis, MN  
55402 (US).
- (81) Designated States (unless otherwise indicated, for every  
kind of national protection available): **AE AG AL AM,**  
**AT AU AZ BA BB BG BR BW BY BZ CA CH CN,**  
**CO CR CU CZ DE DK DM DZ EC EE EG ES FI,**  
**GB GD GE GH GM HR HU ID IL IN IS JP KE,**  
**KG KP KR KZ LC LK LR LS LT LU LV MA MD,**  
**MG MK MN MW MX MZ NA NI NO NZ OM PG,**  
**PH PL PT RO RU SC SD SE SG SK SL SY TJ TM,**  
**TN TR TT TZ UA UG US UZ VC VN YU ZA ZM,**  
**ZW.**
- (84) Designated States (unless otherwise indicated, for every  
kind of regional protection available): **ARIPO (BW, GH,**  
**GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,**  
**ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),**  
**European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,**  
**FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI,**  
**SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,**  
**GW, ML, MR, NE, SN, TD, TG).**
- Published:**  
— without international search report and to be republished  
upon receipt of that report
- For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.*



**WO 2005/028679 A2**

(54) Title: **METHOD AND KIT FOR IDENTIFYING VANCOMYCIN-RESISTANT ENTEROCOCCUS**

(57) Abstract: The invention provides a method to process samples for DNA detection and a method to identify the vancomycin resistance gene status of an organism.

**BEST AVAILABLE COPY**

## METHOD AND KIT FOR IDENTIFYING VANCOMYCIN-RESISTANT ENTEROCOCCUS

5

### Cross-Reference to Related Applications

This application is a continuation-in-part of U.S. application Serial No. 10/661,094, filed September 12, 2003, the disclosure of which is incorporated by reference herein.

10

### Background of the Invention

Three pressures face the routine microbiology laboratory: increasing specimen numbers, principally for infection control purposes; a need to report prompt results; and shrinking budgets. Standard culture screening protocols typically require 3-5 days to issue final results (Sahm et al., 1997; Van Horn et al., 1996) because bacterial identification requires conventional biochemical tests. Other types of assays have been developed into more rapid diagnostic tools, e.g., immunoassays, including radioimmunoassays, enzyme-linked immunoassays, and latex agglutination and immunoblotting assays. Moreover, polynucleotide-based assays are rapidly gaining popularity in clinical laboratory practice.

For example, nucleic acid hybridization assays have been developed to detect microorganisms, and more recent advances in signal and target amplification have introduced the era of molecular diagnostics based on the use of oligonucleotide probes. Generally, a probe is a single-stranded polynucleotide having some degree of complementarity with a nucleic acid sequence that is to be detected ("target sequence"). A double-stranded nucleic acid hybrid between the probe and the target sequence results if the target sequence is contacted under hybridization-promoting conditions with a probe having a sufficient number of contiguous bases complementary to the target sequence. DNA/DNA, RNA/DNA or RNA/RNA hybrids may thus be formed under appropriate conditions. Probes commonly are labeled with a detectable moiety such as a radioisotope, a ligand, or a colorimetric, fluorometric or chemiluminescent moiety to facilitate the detection of hybrids.

Enterococci have been recognized as an important cause of nosocomial infection for the past two decades (Murray, 1990). High level resistance to vancomycin in enterococci is carried on a transposable gene cassette (Quintiliani et al., 1996; Evers et al., 1996; Arthur et al., 1992) with two distinct phenotypes; 5 VanA (inducible resistance to both vancomycin and teicoplanin) and VanB (inducible resistance to vancomycin, but not teicoplanin). Studies linked higher morbidity and mortality rates to vancomycin resistance enterococci (VRE) infections and the number of isolates of enterococci resistant to vancomycin has increased dramatically in the last decade (Centers for Disease Control, 1993; 10 Bemston et al., (1998). Because the organism can be transferred by nosocomial spread and remains viable in the environment, VRE containment protocols that include surveillance have been established (Centers for Disease Control, 1995). Nevertheless, these surveillance programs can be time-consuming, as culture requires 72-96 hours, as well as costly, to the clinical microbiology laboratory. 15 Moreover, culture for VRE has a documented sensitivity of 58%, which is problematic.

A number of recent reports have focused on applying polymerase chain reaction (PCR) technology to detect VRE in a more timely manner. Some of these assays have been performed directly on clinical specimens (Petrich et al., 20 1999; Satake et al., 1997), while others have used an enrichment step in an overnight broth (Satake et al., 1997) or on selective media (Sahm et al., 1997). To minimize turnaround time, the ideal protocol would be PCR performed directly on the specimen. Nevertheless, one issue with direct amplification is that specimen preparation remains technically demanding and may not be 25 suitable for the routine technologist (Petrich et al., 1999). A second issue is that a cultured specimen may still be required for identification at the species level and for epidemiologic typing. An enrichment culture of the VRE specimen would provide a simple specimen preparation, presumably fewer amplification inhibitors, and a cultured isolate available for subsequent confirmation.

30 One example of a polynucleotide-based assay for VRE is described in Petrich et al. (1999; 2000). In that assay, denatured biotinylated PCR amplicons are mixed with a fluorescein-labeled (FITC) detector probe and the mixture transferred to streptavidin-coated microtiter wells. After incubation and washing, a horseradish peroxidase (HRP)-conjugated antibody specific for FITC

is added to detect VRE-specific amplicons.

However, there is a continuing need for rapid and accurate assays to detect VRE in patient samples.

5

### Summary of the Invention

The present invention relates to polynucleotide-based methods, compositions, kits and devices that can be used to detect the *vanA* gene or the *vanB* gene, which genes are each associated with vancomycin resistance of microorganisms. As described hereinbelow, a rapid real time PCR for the  
10 detection of both *vanA* and *vanB* positive enterococci was developed. In particular, primers and fluorescent probes were designed that were specific for the *vanA* gene and all known *vanB* genes. Peri-rectal swabs for routine surveillance were cultured, then the swabs resuspended in 1 mL of phosphate buffer saline (PBS). 305 swabs were tested in total. An extract prepared from  
15 the PBS samples then was tested in a real time assay for both genes, and the PCR data compared to the current gold standard of culture. Using ATCC strains, the primers were shown to demonstrate specificity for each gene type. Moreover, the limit of detection was determined to be 80 per reaction (40 cfu/ $\mu$ L) for the *vanA* gene and 8 per reaction (4 cfu/ $\mu$ L) for the *vanB* gene. The  
20 PCR based assay detected 12 positive specimens that were not identified by standard culture. Of these, 8 were positive for the *vanA* gene and 4 were positive for the *vanB* gene, and were later identified as true positives by enriched culture (i.e., a liquid overnight culture, for example, in tryptic soy broth, inoculated with PBS contacted with the swab, and subsequently plated on solid medium  
25 containing vancomycin and bile esulin). The overall sensitivity and specificity of this rapid assay is 93.4% and 99.1%, respectively.

Thus, a marked increase in detection sensitivity in comparison to culture was observed using the rapid nucleic acid amplification-based assay described herein. The assay therefore allows the rapid detection of *vanA* and *vanB* genes,  
30 e.g., in the same day the sample is obtained which is very useful in clinical laboratories and hospitals to identify vancomycin resistance genes. In particular, the method of the invention can identify the *vanA* or *vanB* status of a patient and can lead to the appropriate choice of antibiotics to treat an infection, thereby reducing the occurrence of antibiotic resistance. The method also reduces the

amount of time a patient has contact with others before their *vanA* or *vanB* status is known, and can result in speedier discharge of patients to nursing and extended care facilities, which may require the current *vanA* or *vanB* status of those patients.

- 5           The invention includes a method to detect the presence of a *vanA* gene and/or a *vanB* gene in a biological sample. In one embodiment, the sample is a physiological sample such as a peri-rectal sample. In one embodiment, the sample is from a culture, e.g., a portion of or an individual colony including those from an enriched culture, or from a liquid culture. The method includes
- 10   providing, e.g., by extracting, nucleic acid from a biological sample of a mammal at risk of having, e.g., by exposure to a mammal having a bacterial infection, or suspected of having a bacterial infection, adding one or more reagents to the nucleic acid sample or a portion thereof, e.g., one or more oligonucleotide primers, to yield an amplification reaction mixture, and
- 15   subjecting the amplification reaction mixture or a portion thereof to conditions effective to amplify *vanA* and/or *vanB* sequences. In one embodiment, the amplification reaction mixture includes two or more oligonucleotide primers specific for one or more different genes in a single reaction vessel.
- Alternatively, a portion of the nucleic acid sample is added to two or more
- 20   reaction vessels, and amplification reactions for one or more different genes conducted in those vessels. In one embodiment, separate amplification reactions are conducted, one for the *vanA* gene and another for the *vanB* gene. The resulting amplified reactions may then be combined prior to contact with a *vanA* or *vanB*-specific probe hybridization. In another embodiment, a single reaction
- 25   vessel is employed to conduct an amplification reaction for both the *vanA* gene and the *vanB* gene. In another embodiment, the *van*-specific reaction or a separate reaction can include control primers and/or a control probe.

- Optionally, the amplified mixture or the amplification reaction mixture is contacted with at least one probe, e.g., a *vanA*-specific probe and/or a *vanB*-
- 30   specific probe, and optionally one or more reagents, which under appropriate conditions, preferably high stringency conditions, are effective to hybridize a *vanA*-specific probe and/or a *vanB*-specific probe to target DNA, i.e., to form a hybrid between the target DNA and sequences in the probe which are complementary thereto, and the presence or amount of hybridized probe detected

or determined, e. g., at one or more time points. In one preferred embodiment, the one or more probes are labeled with a detectable moiety or a moiety capable of detection. In one embodiment, a *vanA*-specific probe is labeled. In another embodiment, a *vanB*-specific probe is labeled. In another embodiment, a *vanA*-specific probe and a *vanB*-specific probe are labeled, e.g., each with a one or more different labels. In one embodiment, the amplified mixture is contacted with at least one probe and one or more reagents, to yield a hybridization reaction mixture. For instance, a portion of the amplification reaction may be added to a reaction vessel and one or more probes and one or more reagents added to the vessel, or a portion of the amplification reaction may be added to at least two reaction vessels and one or more probes and one or more reagents added to each of those vessels. Alternatively, an amplification reaction or a portion thereof is added to one or more probes and one or more reagents in a reaction vessel.

Thus, in one embodiment, the amplification reaction includes a nucleic acid sample and one or more *vanA*-specific primers and one or more *vanB*-specific primers, and the resulting amplified mixture is contacted with at least two probes including a *vanA*-specific probe and a *vanB*-specific probe under conditions, preferably high stringency conditions, effective to hybridize the probes to their respective target DNAs, i.e., to form a hybrid between the target DNA and sequences in each probe which are complementary thereto. In another embodiment, separate *vanA*-specific and *vanB*-specific amplification reactions are conducted, then the reactions or a portion thereof are combined, and a *vanA*-specific and *vanB*-specific hybridization reaction conducted in a single vessel. Accordingly, in one embodiment, the method comprises contacting a biological sample comprising nucleic acid or a portion thereof with one or more *vanA*-specific oligonucleotide primers under conditions effective to amplify *vanA* sequences. Previously, concurrently or subsequently, e.g., in the same or a different reaction vessel, a nucleic acid sample or a portion thereof is contacted with one or more *vanB*-specific oligonucleotide primers under conditions effective to amplify *vanB* sequences. In one embodiment, the amplified sample is contacted with one or more *vanA*-specific or *vanB*-specific oligonucleotide probes under high stringency hybridization conditions effective to form a hybrid between the oligonucleotide probes and *vanA* and/or *vanB* amplified nucleic

acid, and the presence or amount of hybrid formation detected or determined.

Hence, in one embodiment, separate *vanA*-specific and *vanB*-specific amplification and hybridization reactions are conducted.

In yet another embodiment, the amplification reaction includes a nucleic acid sample, one or more *vanA*-specific primers and one or more *vanB*-specific primers, and one or more probes, e.g., at least two probes including a *vanA*-specific probe and a *vanB*-specific probe, which is subjected to conditions effective to amplify *vanA*-specific and *vanB*-specific sequences and to hybridize the probes to their respective target DNAs. Then the presence or amount of hybrid formation detected or determined. In one preferred embodiment, the one or more probes are labeled with a detectable moiety or a moiety capable of detection. In one embodiment, a *vanA*-specific probe is labeled. In another embodiment, a *vanB*-specific probe is labeled. In another embodiment, a *vanA*-specific probe and a *vanB*-specific probe are labeled, e.g., each with a one or more different labels. Exemplary conditions for amplification, or amplification and hybridization, include about 55°C for about 2 minutes, about 95°C for 10 minutes, followed by about 45 cycles of about 95°C for about 15 seconds and about 60°C for about 1 minute. Thus, by probing an amplified sample with probes towards the *vanA* gene and the *vanB* gene, a single sample can be employed to detect both antibiotic resistance genes.

In one embodiment, the oligonucleotides of the invention include sequences substantially corresponding to nucleotides 851 to 868 of the *vanA* gene (SEQ ID NO:2; an exemplary *vanA* gene has SEQ ID NO:1 from *E. faecium* pIP816 gi 43335, also see Figure 1, Accession No. X56895 which corresponds to SEQ ID NO:11), or the complement thereof, or a portion thereof; sequences substantially corresponding to nucleotides 870 to 896 of the *vanA* gene (SEQ ID NO:3), the complement thereof, or a portion thereof; sequences substantially corresponding to nucleotides 898 to 917 of the *vanA* gene (SEQ ID NO:4), the complement thereof, or a portion thereof; sequences substantially corresponding to nucleotides 387 to 404 of the *vanB* gene (SEQ ID NO:6, an exemplary *vanB* gene has SEQ ID NO:5 which corresponds to Accession No. U00456, also shown in Figure 2), the complement thereof, or a portion thereof; sequences substantially corresponding to nucleotides 406 to 423 of the *vanB* gene (SEQ ID NO:7), the complement thereof, or a portion thereof; or sequences

substantially corresponding to nucleotides 426 to 446 of the *vanB* gene (SEQ ID NO:8 or SEQ ID NO:9), the complement thereof, or a portion thereof. In one embodiment, the oligonucleotide primers include sequences substantially corresponding to nucleotides 851 to 868 or 898 to 917 of the *vanA* gene, the complement thereof or a portion thereof. In one embodiment, the oligonucleotide primers include sequences substantially corresponding to nucleotides 387 to 404 or 426 to 446 of the *vanB* gene, the complement thereof, or a portion thereof. In one embodiment, the oligonucleotide probe includes sequences substantially corresponding to nucleotides 870 to 896 of the *vanA* gene, the complement thereof, or a portion thereof, or sequences substantially corresponding to nucleotides 406 to 423 of the *vanB* gene, the complement thereof, or a portion thereof. Preferably, the probe is labeled, e.g., with one or more labels such as a fluorescent or chemiluminescent label. In one embodiment, the probes for *vanA* sequences and *vanB* sequences have different labels. Optionally, one or more non-*vanA* gene or non-*vanB* gene probes, may be employed, e.g., to identify the microorganism in the sample and/or to confirm that sufficient DNA was present in the sample to detect the *vanA* and/or *vanB* gene (an internal control). In one embodiment, the  $T_m$  of an oligonucleotide employed as a probe is at least about 10°C higher than the  $T_m$  of an oligonucleotide employed as a primer in an amplification/hybridization reaction

In another embodiment of the invention, one or more *vanA*-specific and/or *vanB*-specific oligonucleotide probes are employed with a sample which does not contain amplified nucleic acid. The method includes contacting a sample comprising nucleic acid with at least one *vanA*-specific oligonucleotide probe and/or at least one *vanB*-specific oligonucleotide probe under high stringency hybridization conditions effective to form a hybrid between each probe and the target nucleic acid, and the presence or amount of hybrid formation detected or determined. Oligonucleotides useful in this embodiment of the invention include those with sequences substantially corresponding to nucleotides 851 to 917 or any contiguous portion thereof greater than about 15 nucleotides in length, e.g., nucleotides 851 to 868, 870 to 896, or 898 to 917, of the *vanA* gene, the complement thereof, or a portion thereof, or nucleotides 387 to 446 or any contiguous portion thereof greater than about 15 nucleotides in

length, e.g., nucleotides 387 to 404, 406 to 423, or 426 to 446, of the *vanB* gene, the complement thereof, or a portion thereof.

The invention also includes one or more oligonucleotides. The oligonucleotides include one or more of an oligonucleotide substantially  
5 corresponding to nucleotides 851 to 868 of the *vanA* gene, the complement thereof, or a portion thereof, an oligonucleotide substantially corresponding to nucleotides 898 to 917 of the *vanA* gene, the complement thereof, or a portion thereof, an oligonucleotide substantially corresponding to nucleotides 870 to 896 of the *vanA* gene, the complement thereof or a portion thereof, an  
10 oligonucleotide substantially corresponding to nucleotides 387 to 404 of the *vanB* gene, the complement thereof, or a portion thereof, an oligonucleotide substantially corresponding to nucleotides 406 to 423 of the *vanB* gene, the complement thereof, or a portion thereof, and an oligonucleotide substantially corresponding to nucleotides 426 to 446 of the *vanB* gene, the complement  
15 thereof, or a portion thereof. Each oligonucleotide anneals to *vanA* and/or *vanB* DNA under stringent hybridization conditions. In one embodiment, the invention includes an oligonucleotide mix including an oligonucleotide corresponding to nucleotides 851 to 868 of the *vanA* gene, or a portion thereof, and an oligonucleotide corresponding to the complement of nucleotides 898 to  
20 917 of the *vanA* gene, or a portion thereof. In one embodiment, the invention includes an oligonucleotide mix including an oligonucleotide corresponding to nucleotides 387 to 404 of the *vanB* gene, or a portion thereof, and an oligonucleotide corresponding to the complement of nucleotides 426 to 446 of the *vanB* gene, or a portion thereof.

25 The invention further includes a probe composition. The composition includes one or more oligonucleotide substantially corresponding to nucleotides 870 to 896 of the *vanA* gene, the complement thereof, or a portion thereof, or an oligonucleotide substantially corresponding to nucleotides 406 to 423 of the *vanB* gene, the complement thereof, or a portion thereof.

30 The invention further includes a kit with primers and/or probes useful to amplify and/or detect the *vanA* gene and/or the *vanB* gene in a test sample. The kit includes one or more oligonucleotide comprising sequences corresponding to nucleotides 870 to 896 of the *vanA* gene, the complement thereof, or a portion thereof, an oligonucleotide comprising sequences corresponding to nucleotides

851 to 868 of the *vanA* gene, the complement thereof, or a portion thereof, and an oligonucleotide comprising sequences corresponding to nucleotides 898 to 917 of the *vanA* gene, the complement thereof, or a portion thereof, wherein each oligonucleotide anneals, e.g., under stringent hybridization conditions, to *vanA* DNA. The kit optionally includes other probes, e.g., non-*vanA* probes, for instance, primers or probes useful to amplify or detect other genes, including other drug resistance genes.

In one embodiment, the kit includes one or more of an oligonucleotide comprising sequences substantially corresponding to nucleotides 387 to 404 of the *vanB* gene, the complement thereof, or a portion thereof, an oligonucleotide comprising sequences substantially corresponding to nucleotides 406 to 423 of the *vanB* gene, the complement thereof, or a portion thereof, or an oligonucleotide comprising sequences substantially corresponding to nucleotides 426 to 446 of the *vanB* gene, the complement thereof, or a portion thereof. The kit optionally includes other probes, e.g., non-*vanB* probes, for instance, primers or probes useful to amplify or detect other genes, including other drug resistance genes.

#### **Brief Description of the Figures**

Figure 1. A representative *vanA* sequence (SEQ ID NO:1). Underlining shows the position of an exemplary forward primer, probe and reverse primer.

Figure 2. Alignment of 8 *vanB* sequences (SEQ ID NOs:10-16 and 5, respectively), individual sequences (SEQ ID NOs:10-16 and 5), and a consensus (majority) sequence (SEQ ID NO:17).

Figure 3. Organisms tested for specificity of *vanA* and *vanB* primers and probes.

Figure 4. An exemplary sequence (SEQ ID NO:18) useful as an internal control and positions of exemplary primers and a probe (SEQ ID Nos. 19-21).

#### **Detailed Description of the Invention**

##### **Definitions**

As used herein, the following terms have the given meanings unless expressly stated to the contrary.

A "nucleotide" is a subunit of a nucleic acid comprising a purine or pyrimidine base group, a 5-carbon sugar and a phosphate group. The 5-carbon sugar found in RNA is ribose. In DNA, the 5-carbon sugar is 2'-deoxyribose. The term also includes analogs of such subunits, such as a methoxy group (MeO) at the 2' position of ribose.

An "oligonucleotide" is a polynucleotide having two or more nucleotide subunits covalently joined together. Oligonucleotides are generally about 10 to about 100 nucleotides in length, or more preferably 10 to 50 nucleotides in length. The sugar groups of the nucleotide subunits may be ribose, deoxyribose, or modified derivatives thereof. The nucleotide subunits may be joined by linkages such as phosphodiester linkages, modified linkages or by non-nucleotide moieties that do not prevent hybridization of the oligonucleotide to its complementary target nucleotide sequence. Modified linkages include those in which a standard phosphodiester linkage is replaced with a different linkage, such as a phosphorothioate linkage, a methylphosphonate linkage, or a neutral peptide linkage. Nitrogenous base analogs also may be components of oligonucleotides in accordance with the invention. Ordinarily, oligonucleotides will be synthesized by organic chemical methods and will be single-stranded unless specified otherwise. Oligonucleotides can be labeled with a detectable label.

A "target nucleic acid" is a nucleic acid comprising a target nucleic acid sequence.

A "target nucleic acid sequence," "target nucleotide sequence" or "target sequence" is a specific deoxyribonucleotide or ribonucleotide sequence that can be hybridized by an oligonucleotide. For instance, a "target nucleic acid sequence region" of bacteria in the *Enterococcus* genus refers to a nucleic acid sequence present in nucleic acid or a sequence complementary thereto found in *Enterococcus* bacteria, which is not present in nucleic acids of other species. Nucleic acids having nucleotide sequences complementary to a target sequence may be generated by target amplification techniques such as polymerase chain reaction (PCR).

A "primer" is a single-stranded polyoligonucleotide that combines with a complementary single-stranded target to form a double-stranded hybrid, which

primer in the presence of a polymerase and appropriate reagents and conditions, results in nucleic acid synthesis.

A "probe" is a single-stranded polynucleotide that combines with a complementary single-stranded target polynucleotide to form a double-stranded hybrid. A probe may be an oligonucleotide or a nucleotide polymer, and may contain a detectable moiety which can be attached to the end(s) of the probe or can be internal to the sequence of the probe. The nucleotides which combine with the target polynucleotide need not be strictly contiguous as may be the case with a detectable moiety internal to the sequence of the probe.

10 A "detectable moiety" is a label molecule attached to, or synthesized as part of, a polynucleotide probe. This molecule should be uniquely detectable and will allow the probe to be detected as a result. These detectable moieties include but are not limited to radioisotopes, colorimetric, fluorometric or chemiluminescent molecules, enzymes, haptens, redox-active electron transfer  
15 moieties such as transition metal complexes, metal labels such as silver or gold particles, or even unique oligonucleotide sequences.

A "hybrid" is the complex formed between two single-stranded polynucleotide sequences by Watson-Crick base pairings or non-canonical base pairings between the complementary bases. By "nucleic acid hybrid" or  
20 "probe:target duplex" is meant a structure that is a double-stranded, hydrogen-bonded structure, preferably 10 to 100 nucleotides in length, more preferably 14 to 50 nucleotides in length. The structure is sufficiently stable to be detected by means such as chemiluminescent or fluorescent light detection, colorimetry, autoradiography, electrochemical analysis or gel electrophoresis. Such hybrids  
25 include RNA:RNA, RNA:DNA, or DNA:DNA duplex molecules.

"Hybridization" is the process by which two complementary strands of polynucleotide combine to form a stable double-stranded structure ("hybrid complementarity" is a property conferred by the base sequence of a single strand of DNA or RNA which may form a hybrid or double-stranded DNA:DNA,  
30 RNA:RNA or DNA:RNA through hydrogen bonding between Watson-Crick base pairs on the respective strands). Adenine (A) ordinarily complements thymine (T) or uracil (U), while guanine (G) ordinarily complements cytosine (C).

"Stable" means resistant to chemical or biochemical degradation, reaction, decomposition, displacement or modification.

"Stability" means the resistance of a substance to chemical or biochemical degradation, reaction, decomposition, displacement or modification.

5       The term "stringency" is used to describe the temperature and solvent composition existing during hybridization and the subsequent processing steps. Under high stringency conditions only highly complementary nucleic acid hybrids will form; hybrids without a sufficient degree of complementarity will not form. Accordingly, the stringency of the assay conditions determines the  
10       amount of complementarity needed between two polynucleotide strands forming a hybrid. Stringency conditions are chosen to maximize the difference in stability between the hybrid formed with the target and the non-target polynucleotide.

      The term "probe specificity" or "primer specificity" refers to a  
15       characteristic of a probe or primer which describes its ability to distinguish between target and non-target sequences. Probe or primer specificity is dependent on sequence and assay conditions and may be absolute (i.e., the primer or probe can distinguish between nucleic acid from target organisms and any non-target organisms), or it may be functional (i.e., the primer or probe can  
20       distinguish between the nucleic acid from a target organism and any other organism normally present in a particular sample).

      "Polynucleotide" means either RNA or DNA, along with any synthetic nucleotide analogs or other molecules that may be present in the sequence and that do not prevent hybridization of the polynucleotide with a second molecule  
25       having a complementary sequence. The term includes polymers containing analogs of naturally occurring nucleotides and particularly includes analogs having a methoxy group at the 2' position of the ribose (MeO).

      A "biological sample" refers to a sample of material that is to be tested for the presence of microorganisms or nucleic acid thereof. The biological  
30       sample can be obtained from an organism, e.g., it can be a physiological sample, such as one from a human patient, a laboratory mammal such as a mouse, rat, pig, monkey or other member of the primate family, by drawing a blood sample, sputum sample, spinal fluid sample, a urine sample, a rectal swab, a peri-rectal swab, a nasal swab, a throat swab, or a culture of such a sample, e.g., a colony

on a plate or a liquid culture. Ordinarily, the biological sample will contain hybridizable polynucleotides. These polynucleotides may have been released from organisms that comprise the biological sample, or alternatively can be released from the organisms in the sample using techniques such as sonic  
5 disruption or enzymatic or chemical lysis of cells to release polynucleotides so that they are available for amplification with one or more polynucleotide primers or hybridization with a polynucleotide probe.

"T<sub>m</sub>" refers to the temperature at which 50% of the probe or primer is converted from the hybridized to the unhybridized form.

10 One skilled in the art will understand that probes or primers that substantially correspond to a reference sequence or region can vary from that reference sequence or region and still hybridize to the same target nucleic acid sequence. Probes of the present invention substantially correspond to a nucleic acid sequence or region if the percentage of identical bases or the percentage of  
15 perfectly complementary bases between the probe and its target sequence is from 100% to 80% or from 0 base mismatches in a 10 nucleotide target sequence to 2 bases mismatched in a 10 nucleotide target sequence. In one embodiment, the percentage is from 100% to 85%. In another embodiment this percentage is from 90% to 100%; and in yet other embodiments, this percentage is from 95%  
20 to 100%. Probes or primers that substantially correspond to a reference sequence or region include probes or primers having any additions or deletions which do not prevent the probe or primer from having its claimed property, such as being able to preferentially hybridize under high stringency hybridization conditions to its target nucleic acid over non-target nucleic acids.

25 By "sufficiently complementary" or "substantially complementary" is meant nucleic acids having a sufficient amount of contiguous complementary nucleotides to form a hybrid that is stable for detection or to initiate nucleic acid synthesis.

By "anti-sense" is meant a nucleic acid molecule perfectly  
30 complementary to a reference (i.e., sense) nucleic acid molecule.

"RNA and DNA equivalents" refer to RNA and DNA molecules having the same complementary base pair hybridization properties. RNA and DNA equivalents have different sugar groups (i.e., ribose versus deoxyribose), and may differ by the presence of uracil in RNA and thymine in DNA. The

difference between RNA and DNA equivalents do not contribute to differences in substantially corresponding nucleic acid sequences because the equivalents have the same degree of complementarity to a particular sequence.

## 5 I. Oligonucleotide Primers and Probes

It is not always necessary to determine the entire nucleic acid sequence of a gene of interest in order to obtain an oligonucleotide primer or probe sequence for that gene or to determine the nucleic acid sequence of that gene from a large number of sources in order to detect heterogeneity. Once a sequence  
10 is available for a gene of interest or a portion thereof, the following guidelines are useful for designing primers or probes with desired characteristics.

First, the stability of the oligonucleotide:target polynucleotide hybrid is chosen to be compatible with the assay conditions. This may be accomplished by avoiding long A and T rich sequences, by terminating the hybrids with G:C  
15 base pairs and by designing the probe in such a way that the  $T_m$  will be appropriate for standard conditions to be employed in the assay (amplification or hybridization). The nucleotide sequence of the primer or probe should be chosen so that the length and % G and % C result in a probe having a  $T_m$  about 2 to  
20  $10^\circ\text{C}$  higher than the temperature at which the final assay is performed. The base composition of the primer or probe is significant because G:C base pairs exhibit greater thermal stability when compared with A:T base pairs. Thus, hybrids involving complementary polynucleotides having a high G:C content are generally stable at higher temperatures when compared with hybrids having a lower G:C content.

25 Second, the position at which the primer or probe binds its target polynucleotide is chosen to minimize the stability of hybrids formed between probe:non-target polynucleotides. This may be accomplished by minimizing the length of perfect complementarity with polynucleotides of non-target organisms, by avoiding G:C rich regions of homology with non-target sequences, and by  
30 positioning the primer or probe to span as many destabilizing mismatches as possible. Whether a primer or probe sequence is useful for amplifying or detecting only a specific type of organism or gene depends largely on thermal stability differences between probe:target hybrids and probe:non-target hybrids.

The differences in  $T_m$  should be as large as possible to produce highly specific primers and probes.

The length of the target polynucleotide sequence and the corresponding length of the primer or probe sequence also are important factors to be  
5 considered when designing a primer or probe. While it is possible for polynucleotides that are not perfectly complementary to hybridize to each other, the longest stretch of perfectly homologous base sequence is ordinarily the primary determinant of hybrid stability.

Third, regions which are known to form strong internal structures  
10 inhibitory to hybridization of a primer or probe are less preferred as targets. Primers or probes having extensive self-complementarity also should be avoided.

Once a presumptive unique sequence has been identified, corresponding oligonucleotides are produced. Defined oligonucleotides that can be used to  
15 practice the invention can be produced by any of several well-known methods, including automated solid-phase chemical synthesis using phosphoramidite precursors (Barone et al., 1984). Other well-known methods for construction of synthetic oligonucleotides may, of course, be employed (see Sambrook et al., 1989). All of the oligonucleotides of the present invention may be modified  
20 with chemical groups to enhance their performance. Backbone-modified oligonucleotides, such as those having phosphorothioate or methylphosphonate groups, are examples of analogs that can be used in conjunction with oligonucleotides of the present invention. These modifications render the oligonucleotides resistant to the nucleolytic activity of certain polymerases or to  
25 nuclease enzymes. Other analogs that can be incorporated into the structures of the oligonucleotides include peptide nucleic acids, or "PNAs." The PNAs are compounds comprising ligands linked to a peptide backbone rather than to a phosphodiester backbone. Representative ligands include either the four main naturally occurring DNA bases (i.e., thymine, cytosine, adenine or guanine) or  
30 other naturally occurring nucleobases (e.g., inosine, uracil, 5-methylcytosine or thiouracil) or artificial bases (e.g., bromothymine, azaadenines or azaguanines, etc.) attached to a peptide backbone through a suitable linker. PNAs are able to bind complementary ssDNA and RNA strands. Methods for making and using PNAs are disclosed in U.S. Patent No. 5,539,082. Another type of modification

that can be used to make oligonucleotides having the sequences described herein involves the use of non-nucleotide linkers (e.g., see U.S. Patent No. 6,031,091) between nucleotides in the nucleic acid chain which do not interfere with hybridization or optionally elongation of a primer.

5 Yet other analogs include those which increase the binding affinity of a probe to a target nucleic acid and/or increase the rate of binding of the probe to the target nucleic acid relative to a probe without the analog. Such analogs include those with a modification (substitution) at the 2' position of a ribofuranosyl nucleotide. Analogs having a modification at the 2' position of the  
10 ribose are one embodiment. Other substitutions at the 2' position of the sugar are expected to have similar properties so long as the substitution is not so large as to cause steric inhibition of hybridization. Thus, hybridization assay probes can be designed to contain modified nucleotides which, alone or in combination, may have the advantage of increasing the rate of target-specific hybridization.

15 Preferably, probes are labeled. Essentially any labeling and detection system that can be used for monitoring specific nucleic acid hybridization can be used in conjunction with the probes disclosed herein when a labeled probe is desired. Included among the collection of useful labels are: radiolabels, enzymes, haptens, linked oligonucleotides, colorimetric, fluorometric, e.g., 6-carboxyfluorescein (FAM), carboxytetramethylrhodamine (TAMRA), or VIC  
20 (Applied Biosystems), or chemiluminescent molecules, and redox-active moieties that are amenable to electrochemical detection methods. In one embodiment, probes are labeled at one end with a reporter dye and with a quencher at the other end, e.g., reporters including FAM, 6-tetrachlorofluorescein (TET), MAX (Synthegen), Cy5 (Synthegen), 6-carboxy-X-rhodamine or 5(6)-carboxy-X-rhodamine (ROX), and TAMRA and quenchers  
25 including TAMRA, BHQ (Biosearch Technologies) and QSY (Molecular Probes). Standard isotopic labels that can be used to produce labeled oligonucleotides include  $^3\text{H}$ ,  $^{35}\text{S}$ ,  $^{32}\text{P}$ ,  $^{125}\text{I}$ ,  $^{57}\text{Co}$  and  $^{14}\text{C}$ . When using  
30 radiolabeled probes, hybrids can be detected by autoradiography, scintillation counting or gamma counting.

Non-isotopic materials can also be used for labeling oligonucleotide probes. These non-isotopic labels can be positioned internally or at a terminus of the oligonucleotide probe. Modified nucleotides can be incorporated

enzymatically or chemically with modifications of the probe being performed during or after probe synthesis, for example, by the use of non-nucleotide linker groups. Non-isotopic labels include colorimetric molecules, fluorescent molecules, chemiluminescent molecules, enzymes, cofactors, enzyme substrates, haptens or other ligands. For instance, U.S. Patent No. 5,998,135 discloses yet another method that can be used for labeling and detecting probes using fluorimetry to detect fluorescence emission from lanthanide metal labels disposed on probes, where the emission from these labels becomes enhanced when it is in close proximity to an energy transfer partner. Exemplary electrochemical labeling and detection approaches are disclosed in U.S. Patent Nos. 5,591,578 and 5,770,369, and PCT/US98/12082, the disclosures of which are hereby incorporated by reference. Redox active moieties useful as electrochemical labels include transition metals such as Cd, Mg, Cu, Co, Pd, Zn, Fe and Ru. Indeed, any number of different non-isotopic labels can be used for preparing labeled oligonucleotides in accordance with the invention. For example, a probe may contain more than one label.

Alternative procedures for detecting particular genes can be carried out using either labeled probes or unlabeled probes. For example, hybridization assay methods that do not rely on the use of a labeled probe are disclosed in U.S. Patent No. 5,945,286 which describes immobilization of unlabeled oligonucleotide probe analogs made of peptide PNAs, and detectably labeled intercalating molecules which can bind double-stranded PNA probe/target nucleic acid duplexes. In these procedures, as well as in certain electrochemical detection procedures, such as those disclosed in PCT/US98/12082, PCT/US98/12430 and PCT/US97/20014, the oligonucleotide probe is not required to harbor a detectable label.

Nucleic acid primers and probes specific for a gene of interest, such as a drug resistance gene, optionally in combination with one or more probes specific for a group of organisms, or a universal bacterial probe, find use in an assay to detect the presence of the gene of interest in nucleic acid from a biological sample and optionally to identify a group of organisms and/or to ensure that the nucleic acid in the sample is adequate to detect the gene of interest (i.e., an internal control). For instance, in one embodiment of the invention, a plurality of primers and/or probes specific for the *vanA* gene and the *vanB* gene may be

employed to detect whether a biological sample contains *vanA*<sup>+</sup> or *vanA*<sup>-</sup> organisms, as well as *vanB*<sup>+</sup> or *vanB*<sup>-</sup> organisms.

## II. Antibiotic Resistance Gene Primers and Probes

Antimicrobial resistance complicates treatment and often leads to therapeutic failures. Furthermore, overuse of antibiotics inevitably leads to the emergence of bacterial resistance. Besides the rapid identification of negative clinical specimens with DNA-based tests for bacterial detection and the identification of the presence of a pathogen in the positive specimens, the clinician also needs timely information about the ability of the bacterial pathogen to resist antibiotic treatments. Since the sequence from many common bacterial antibiotic resistance genes is available from data banks, the sequence from a portion or from the entire gene is employed to design specific oligonucleotides which will be used as a basis for the development of rapid DNA-based tests.

*VanA* and *vanB* sequences and structurally and/or functionally related sequences from a collection of organisms were aligned to identify candidate conserved sequences that could be used to distinguish *vanA*<sup>+</sup> and/or *vanB*<sup>+</sup> organisms from *vanA*<sup>-</sup> and/or *vanB*<sup>-</sup> organisms. Thus, by examining partial or complete sequences of *vanA*<sup>+</sup> and/or *vanB*<sup>+</sup> genes of various organisms, aligning those sequences with structurally and/or functionally related sequences to reveal areas of maximum homology and areas of sequence variation, *vanA* and/or *vanB* sequences can be identified that are conserved among *vanA* and/or *vanB* genes but exhibit mismatches with structurally and/or functionally related genes. Based on such considerations, the following regions of the *vanA* gene were chosen to prepare oligonucleotides: nucleotides 851 to 868, nucleotides 870 to 896, and nucleotides 898 to 917 of the *vanA* gene having SEQ ID NO:1. Likewise, the following regions of the *vanB* gene were chosen: nucleotides 387 to 404, nucleotides 406 to 423, and nucleotides 426 to 446 of the *vanB* gene having SEQ ID NO:5. Such conserved sequences are then tested against a panel of *vanA* and/or *vanB* standards and bacterial lysates to verify their utility as primers and/or probes under laboratory conditions. In particular, primers and probes that preferentially anneal to a nucleic acid target region and can initiate nucleic acid synthesis and/or form a detectable duplex that indicates the presence of the *vanA* gene or *vanB* gene, are chosen for polynucleotide-based diagnostic assays.

Preferred methods for detecting the presence of the *vanA* or *vanB* gene, include the step of contacting a test sample with at least two oligonucleotide primers under conditions that preferentially amplify *vanA* and/or *vanB* sequences. Alternatively, a test sample is contacted under high stringency hybridization conditions with at least one oligonucleotide probe that preferentially hybridizes to the *vanA* and/or *vanB* gene.

While oligonucleotide probes of different lengths and base composition may be used for detecting the *vanA* gene or the *vanB* gene, preferred oligonucleotides have lengths from 15 up to 40 nucleotides and are sufficiently homologous to the target nucleic acid to permit amplification of a *vanA* or *vanB* template and/or hybridization to such a template under high stringency conditions. However, the specific sequences described herein also may be provided in a nucleic acid cloning vector or transcript or other longer nucleic acid and still can be used for amplifying or detecting the *vanA* gene or the *vanB* gene, i.e., the probes may include sequences unrelated to the *vanA* or *vanB* gene, for instance at the 5' end, the 3' end, or both the 5' and 3' ends. Likewise, primers may include sequences unrelated to the *vanA* gene and/or the *vanB* gene, e.g., at the 5' end. Preferred primers and probes have sequences of up to 40 nucleotides in length and preferably have at least 17 contiguous nucleotides corresponding to sequences in the *vanA* gene or the *vanB* gene, or the complement thereof. Preferred oligonucleotide sequences include RNA and DNA equivalents, and may include at least one nucleotide analog.

The primers and probes are tested against synthetic targets as well as tested against biological samples, in an amplification and/or hybridization reaction so as to detect the *vanA* gene or the *vanB* gene. In one method of determining whether a biological sample contains *vanA* or *vanB* gene sequences, nucleic acids are released from bacterial cells by addition of a lysing agent, e.g., a detergent, or by other known methods for disrupting cells including the use of enzymes, osmotic shock, heat, chemical treatment, and vortexing, for instance, with glass beads, or sonic disruption, for example according to the method disclosed in U.S. Patent No. 5,374,522. Methods suitable for liberating nucleic acids from cells which can then be subjected to hybridization methods have been described in U.S. Patent No. 5,837,452 and in U.S. Patent No. 5,364,763.

Preferably, the probes specifically hybridize to *vanA* or *vanB* DNA only under conditions of high stringency. Under these conditions only highly complementary nucleic acid hybrids will form (i.e., those having at least 14 out of 17 bases in a contiguous series of bases being complementary). Hybrids will not form in the absence of a sufficient degree of complementarity. Accordingly, the stringency of the assay conditions determines the amount of complementarity needed between two nucleic acid strands forming a hybrid. Stringency is chosen to maximize the difference in stability between the hybrid formed with target nucleic acid and non-target nucleic acid.

In one embodiment, the *vanA* oligonucleotides include SEQ ID NOs: 3, 4 or 5, the complement or a portion thereof, and the *vanB* oligonucleotides include SEQ ID NO:6, 7 or 8, the complement or a portion thereof, which preferentially amplify and/or hybridize to the *vanA* or *vanB* gene, respectively.

### III. Amplification and Hybridization

Amplification or hybridization assays may be performed either in tubes or in microtitration plates having multiple wells. For assays in plates, the wells may be coated with the specific amplification primers or probes and/or control DNAs, and the detection of amplification products or the formation of hybrids may be automated. Hybridization assays may also be performed on a solid substrate.

#### A. Amplification

Cells are subjected to conditions which release polynucleotides from the cells, thus forming an extract. For example, cells may be treated with detergents, base and/or heat denatured. If the base is employed, the mixture is then neutralized with an acidic composition. Then reagents are added to yield an amplification reaction (containing, for example, monovalent ions, detergent, dNTPS, primers, and a polymerase).

For DNA amplification by the widely used PCR (polymerase chain reaction) method, primer pairs may be derived from sequenced DNA fragments from clinical samples or from data bank sequences. Prior to synthesis, the potential primer pairs may be analyzed by using the program Oligo™ 4.0 (National Biosciences) to verify that they are likely candidates for PCR amplifications. A select set of primers can then be tested in PCR or other

amplification-based assays performed directly from a bacterial suspension or a known standard to determine their specificity.

During DNA amplification by PCR, two oligonucleotide primers binding respectively to each strand of the denatured double-stranded target DNA from the bacterial genome are used to amplify exponentially *in vitro* the target DNA by successive thermal cycles allowing denaturation of the DNA, annealing of the primers and synthesis of new targets at each cycle (Persing et al, 1993). An exemplary PCR protocols is as follows. Clinical specimens or bacterial colonies are added directly to the 50  $\mu$ L PCR reaction mixtures containing 50 mM KCl, 10 mM Tris-HCl pH 8.3, 2.5 mM  $MgCl_2$ , 0.4  $\mu$ M of each of the two primers, 200  $\mu$ M of each of the four dNTPs and 1.25 Units of Taq DNA polymerase (Perkin Elmer). PCR reactions are then subjected to thermal cycling (3 minutes at 95°C followed by 30 cycles of 1 second at 95°C and 1 second at 55°C) using a Perkin Elmer 480™ thermal cycle and subsequently analyzed by standard ethidium bromide-stained agarose gel electrophoresis. It is clear that other methods for the detection of specific amplification products, which may be faster and more practical for routine diagnosis, may be used. Such methods may be based on the detection of fluorescence after amplification (e.g. TaqMan™ system from Perkin Elmer or Amplisensor™ from Biotronics) or other labels such as biotin (SHARP Signal™ system, Digene Diagnostics), or liquid or solid phase hybridization with an oligonucleotide probe binding to internal sequences of the specific amplification product, e.g., a labeled probe. Methods based on the detection of fluorescence are very rapid and quantitative, and can be automated. For instance, one of the amplification primers or an internal oligonucleotide probe specific to the amplicon(s) is coupled with the fluorochrome or with any other label. Moreover, methods based on the detection of fluorescence are particularly suitable for diagnostic tests since they are rapid and flexible as fluorochromes emitting different wavelengths are available (Perkin Elmer). Further, a variety of fluorochromes emitting at different wavelengths, each coupled with a specific oligonucleotide linked to a fluorescence quencher which is degraded during amplification, thereby releasing the fluorochrome (e.g., TaqMan™, Perkin Elmer), may be employed.

To assure PCR efficiency, glycerol or dimethyl sulfoxide (DMSO) or other related solvents, can be used to increase the sensitivity of the PCR and to

overcome problems associated with the amplification of target with a high GC content or with strong secondary structures. The concentration ranges for glycerol and DMSO are 5 to 15% (v/v) and 3 to 10% (v/v), respectively. For the PCR reaction mixture, the concentration ranges for the amplification primers and the  $MgCl_2$  are about 0.1 to 1.0 and 1.5 to 3.5 mM, respectively. Modifications of the standard PCR protocol using external and nested primers (i.e., nested PCR) or using more than one primer pair (i.e., multiplex PCR) may also be used (Persing et al, 1993), for instance, to detect simultaneously several genes, including antibiotic resistance genes and genes useful to identify species of bacterial pathogens.

The person skilled in the art of DNA amplification knows the existence of other rapid amplification procedures which include linear amplification procedure, e.g., ligase chain reaction (LCR), transcription-based amplification systems (TAS), self-sustained sequence replication (3SR), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA) and branched DNA (bDNA) (Persing et al, 1993). The scope of this invention is not limited to the use of amplification by PCR, but rather includes the use of any rapid nucleic acid amplification methods or any other procedures which may be used to increase rapidity and sensitivity of the tests. Any oligonucleotides suitable for the amplification of specific nucleic acid sequences by approaches other than PCR and within scope of this invention.

Standard precautions to avoid false positive PCR results should be taken. Methods to inactivate PCR amplification products such as the inactivation by uracil-N-glycosylase may be used to control PCR carryover. For example, in the case of direct amplification from a colony, a portion of the colony may be transferred directly to a 50  $\mu$ L PCR reaction mixture (e.g., containing 50 mM KCl, 10 mM Tris pH 8.3, 2.5 mM  $MgCl_2$ , 0.4  $\mu$ M of each of the two primers, 200  $\mu$ M of each of the four dNTPs and 1.25 Unit of Taq DNA polymerase (Perkin Elmer)) using a plastic rod. For the bacterial suspension, 4  $\mu$ L of a cell suspension may be added to 46  $\mu$ L of the same PCR reaction mixture. For both strategies, the reaction mixture is overlaid with 50  $\mu$ L of mineral oil and PCR amplifications are carried out for instance using an initial denaturation step of 3 minutes at 95°C followed by 30 cycles consisting of a 1 second denaturation step at 95°C and of a 1 second annealing step at 55°C in a Perkin Elmer 480™

thermal cycler. PCR amplification products can be analyzed by standard agarose gel (2%) electrophoresis. Amplification products are visualized in agarose gels containing 2.5 µg/mL of ethidium bromide under UV at 254 nm. The entire PCR assay can be completed in approximately one hour.

5           Alternatively, amplification from bacterial cultures may be performed as described above but using a "hot start" protocol. In that case, an initial reaction mixture containing the target DNA, primers and dNTPs was heated to about 85°C prior to the addition of the other components of the PCR reaction mixture. The final concentration of all reagents was as described above. Subsequently, the  
10       PCR reactions were submitted to thermal cycling and analysis as described above.

          To improve bacterial cell lysis and eliminate the PCR inhibitory effects of clinical specimens, samples may be diluted in lysis buffer containing detergent(s). Subsequently, the lysate is added directly to the PCR reaction  
15       mixture. Heat treatments of the lysates, prior to DNA amplification, using the thermocycler or a microwave oven may also be performed to increase the efficiency of cell lysis.

          PCR has the advantage of being compatible with crude DNA preparations. Thus, samples such as blood, cerebrospinal fluid and sera may be used directly in PCR  
20       assays after a brief heat treatment.

#### B. Hybridization

          In hybridization experiments, oligonucleotides (of a size less than about 100 nucleotides) have some advantages over DNA fragment probes of greater than 100 nucleotides in length for the detection of bacteria such as ease of  
25       preparation in large quantities, consistency in results from batch to batch and chemical stability. The oligonucleotide probes may be derived from either strand of the target duplex DNA. The probes may consist of the bases A, G, C, or T or analogs thereof. In one embodiment, the target DNA is denatured, fixed onto a solid support and hybridized with a DNA probe. Conditions for pre-  
30       hybridization and hybridization can be as follows: (i) pre-hybridization in 1 M NaCl+10% dextran sulfate+1% SDS (sodium dodecyl sulfate)+1 µg/ml salmon sperm DNA at 65°C for 15 minutes, (ii) hybridization in fresh pre-hybridization solution containing the labeled probe at 65°C overnight, and (iii) post-hybridization including washing twice in 3 X SSC containing 1% SDS (1 X SSC

is 0.15 M NaCl, 0.015 M NaCitrate) and twice in 0.1 X SSC containing 0.1% SDS; all washes at 65°C for 15 minutes. For probes labeled with radioactive labels, the detection of hybrids is preferably by autoradiography. For non-radioactive labels, such as probes having colorimetric, fluorescent or chemiluminescent labels, target DNA need not be fixed onto a solid support.

For example, stringent conditions are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate (SSC); 0.1% sodium lauryl sulfate (SDS) at 50°C, or (2) employ a denaturing agent such as formamide during hybridization, e.g., 50% formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is use of 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% sodium dodecylsulfate (SDS), and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1 X to 2 X SSC (20 X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1.0 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. An example of highly stringent wash conditions is 0.15 M NaCl at 72°C for about 15 minutes. An example of stringent wash conditions is a 0.2X SSC wash at 65°C for 15 minutes. Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. For short probes (e.g., about 10 to 50 nucleotides), stringent conditions typically involve salt concentrations of less than about 1.5 M, more preferably about 0.01 to 1.0 M, Na ion concentration (or other salts) at pH 7.0 to 8.3.

Results from an amplification and/or probe hybridization reaction can be inputted into a computer or data processor ("computer"), either manually using a keyboard or directly through an interface from an automated device such as a plate reader, film scanner or luminometer. The computer can sort the positive and negative results for a particular sample to establish a profile be compared

with a look-up table stored in a memory device linked to the computer to associate the profile with results obtained using control organisms in order to determine the presence or absence of a gene of interest in the test organism.

Thus, one or more *vanA* or *vanB* probes can be used to identify the *vanA* or *vanB* status of a sample. Of course, a series of positive and negative control hybridizations can be carried out in parallel to ensure validity of the testing results.

#### IV. Kits of the Invention

A test kit may contain one or more oligonucleotides of the invention, e.g., one or more primers or one or more probes specific for one or more antibiotic resistance genes, e.g., the *vanA* or *vanB* gene, and optionally for particular species of bacterium as well as control primers or probes. The kit is provided in the form of test components and, if present, the probe may be unlabeled or labeled, e.g., labeled with a non-radioactive label. Preferably, if more than one labeled probe is present, each is labeled with a different label. The kit will also optionally include test reagents necessary to perform the amplification reaction, e.g., a polymerase, dNTPs, one or more salts, and/or a buffer, and/or reagents necessary to perform the hybridization reaction, e.g., reagents for pre-hybridization, hybridization, washing steps and/or hybrid detection. The kit may include standard samples to be used as negative and positive controls for each test.

In one embodiment, a test kit includes all reagents and controls to perform DNA amplification assays. Diagnostic kits are adapted for amplification by PCR (or other amplification methods) performed directly either from clinical specimens, or from a bacterial colony. Components required for detection of antibiotic resistance genes, and bacterial identification may be included.

It is understood that the use of the probes and amplification primers described in this invention for bacterial detection and identification is not limited to clinical microbiology applications. In fact, these tests could be used by industries for quality control of food, water, pharmaceutical products or other products requiring microbiological control. These tests could also be applied to detect and identify bacteria in biological samples from organisms other than humans (e.g. other primates, mammals, farm animals and live stocks). These

diagnostic tools could also be very useful for research purposes including clinical trials and epidemiological studies.

#### V. Apparatus Useful for Conducting Hybridization Reactions

Examples of formats that can be used to conduct hybridization reactions include, but are by no means limited to: individual tubes each with a different probe or comprising a plurality of probes; the wells of a 96-well or other multi-well microtiter plate; and a solid support such as a dipstick or a "DNA chip" where polynucleotide probes are immobilized to the support at different addresses in a spaced-apart configuration. Identifying microorganisms and/or the presence of gene(s) of interest advantageously can be performed without requiring any *in vitro* amplification step. Alternatively, an amplification step, may be employed.

According to one approach for conducting hybridization procedures, probes can be labeled with distinguishable labels. More particularly, a single tube, well, or support may include distinct probes that are independently labeled with labels that emit peak energy at different times after generating a light emission. Materials and methods that can be used for making and using distinguishable probes useful in connection with the present invention can be found in U.S. Patent No. 5,756,011. Fluorescent labels that produce light at different wavelengths following excitation represent still other examples of distinguishable labels that can be used in connection with the procedures described herein. In this way, two probes that employ distinguishable labels can be distinguished from each other even though they are combined at the same locus of a testing device. Accordingly, it is possible to combine large numbers of different probes at a single location while still being able to distinguish the results of hybridization for the different probes or sets of probes.

In one embodiment, at least two probes in a single hybridization reaction are labeled with detectable moieties which are distinguishable. The labeled probes are mixed and allowed to hybridize to any nucleic acid contained in the test sample having a sequence sufficiently complementary to the probe sequence to allow hybridization under appropriately selective conditions. One labeling reagents are particularly useful in, although not limited to, a homogeneous assay system in which the presence and quantification of the analytes of interest may be detected and measured without the need for the analyte-bound label to be

physically separated from the unbound label prior to detection. However, such reagents may be used in heterogeneous systems or in combinations of homogeneous and heterogenous assay systems as well.

The compositions and methods provided herein may be utilized in a wide variety of other/related methods (e.g., U.S. Pat. Nos. 5,210,015; 5,487,972; 5,422,253; 5,691,142; 5,719,028; 5,130,238; 5,409,818; 5,554,517; 5,589,332; 5,399,491; 5,480,784; 5,215,899; 5,169,766; 5,194,370; 5,474,916; 5,698,400; 5,656,430; and PCT publication nos. WO 88/10215; WO 92/08800, WO 96/02668; WO 97/19193; WO 97/09444; WO 96/21144; WO 92/22671). Other variations of this assay include 'exponential' cycling reactions such as described in U.S. Pat. No. 5,403,711 (see also U.S. Pat. No. 5,747,255).

Representative examples of further suitable assay formats including any of the above assays which are carried out on solid supports such as dipsticks, magnetic beads, and the like (see generally U.S. Pat. Nos. 5,639,428; 5,635,362; 5,578,270; 5,547,861; 5,514,785; 5,457,027; 5,399,500; 5,369,036; 5,260,025; 5,208,143; 5,204,061; 5,188,937; 5,166,054; 5,139,934; 5,135,847; 5,093,231; 5,073,340; 4,962,024; 4,920,046; 4,904,583; 4,874,710; 4,865,997; 4,861,728; 4,855,240; and 4,847,194).

The invention will be further described by the following non-limiting examples.

### Example 1

#### Materials and Methods

##### Oligonucleotides

##### Primer Sequences

*vanA* forward primer: CCG GTG GCA GCT ACG TTT (SEQ ID NO:2)  
(61% GC content)

*vanA* reverse primer: CAC CGA AGG ATG AGC CTG AA (SEQ ID NO:4) (55% GC content)

*vanA* probe: CCT ATC CTG TTT TTG TTA AGC CGG CGC (SEQ ID NO:3, labeled at the 5' end with 6-FAM and at the 3' end with TAMRA) (57% GC content)

The *vanA* amplicon has a length of 67 bp, a  $T_m$  of 82°C, 55% GC content, and a  $T_a$  of 60.

*vanB* forward primer: CGA CCT CAC AGC CCG AAA (SEQ ID NO:6)

*vanB* reverse primer: CGG CAG GAC AAT ATG ATG GAA (SEQ ID NO:8), or CAG CAG GAC AAT ATG ATG GAA (SEQ ID NO:9)

*vanB* probe: CGC TTG CTC AAT TAA GAT (SEQ ID NO:7, labeled at the 5' end with VIC and at the 3' end with a non-fluorescent quencher MGB)

The sequence for the *vanA* primers was based on the *vanA* gene sequence from GenBank *E. faecium* pIP816 gi 43335 (Figure 1). The sequence for the *vanB* primers was based on a conserved region found in an alignment of 8 known clinical sequences (Figure 2). Generally, oligonucleotide criteria were selected as follows: minimum of 30% and maximum of 80% GC content, preferably about 50% GC content, no repeats, no GC rich 3' end, about 15 to 20 contiguous nucleotides of *vanA* or *vanB*-specific sequences,  $T_m$  of about 59°C, and a maximum 3' consensus match of 7 nucleotides. All sequences selected were then run through BLAST to ensure that there was no cross reactivity with other organisms.

#### Sample Processing and Reaction Conditions

Controls included a 500 µl negative extraction control (sterile RNA/DNA free water) and a 500 µl positive extraction control for each of a *vanA* and a *vanB* bacterial suspension, optionally run in duplicate, a no template control, optionally in duplicate for each set of primers, and optionally an internal control (e.g., using the ABI internal control kit).

Precautions to limit false positives were employed, e.g., the use of separate work areas, dedicated equipment and lab coats, and decontamination, e.g., 10% bleach, sanicloth disinfectant, and UV.

Primers were diluted to 2 µM and probes to 1 µM with sterile RNA/DNA free water. Patient samples were processed before the positive extraction control then the reagent blank was processed. Each patient swab was introduced to a tube with 1 ml PBS, then vortexed. The swab was removed and sediment allowed to form for 5 minutes at room temperature. The samples can be stored at 2-8°C for up to 7 days. 500 µl of a cell lysis solution was added to 500 µl of a patient sample in an eppendorf tube, vortexed, then incubated at 65°C for 15 minutes in a dry heat block. 200 µl of a protein precipitation solution was added to each tube, and vortexed, after which the sample was placed on ice for 5 minutes. The sample was subjected to centrifugation at 14,000 x g for 3

minutes. The supernatant was added to a fresh tube containing 600  $\mu$ l of isopropanol. The tube was inverted several times, then incubated at room temperature for 5 minutes. The mixture was subjected to centrifugation at 14,000 x g for 5 minutes, the resulting supernatant discarded, and residual liquid drained. 600  $\mu$ l of 70% ethanol was added to the pellet, the tube inverted several times, and subsequently subjected to centrifugation at 14,000 x g for 1 minute. The supernatant was discarded and the pellet dried. The dried pellet was resuspended in 20  $\mu$ l of sterile RNA/DNA free water and stored at 2-8°C or less than 0°C.

For each reaction, the following reagents were added and mixed.

	12.5 $\mu$ l	2X ABI Master mix
	3.0 $\mu$ l	sterile water
	2.5 $\mu$ l	forward primer
	2.5 $\mu$ l	reverse primer
15	2.5 $\mu$ l	probe
	<u>2 <math>\mu</math>l</u>	sample
	25 $\mu$ l	

For reaction mixtures for multiple samples, 23  $\mu$ l of a reaction mixture (without added sample) was added to each reaction vessel, e.g., one or more wells of a 96-well plate, then 2  $\mu$ l of a control sample or a DNA sample added. The reaction vessels were then sealed, e.g., by sealing the 96-well plate. Assay conditions included about 55°C for about 2 minutes, about 95°C for 10 minutes, followed by about 45 cycles of about 95°C for about 15 seconds and about 60°C for about 1 minute.

### Results

Currently the gold standard for the detection of VRE is culture. This not only lacks sensitivity but also is time consuming. Time is key in that hospital cost is increased whilst patient status is being determined and that in this time, infected patients can potentially spread the organism to other patients. In particular, knowledge of whether patients carry vancomycin resistance genes is paramount in high-risk units and long term care facilities. Prevention of spread is the key as the resistance genes may be transferred to another bacterium, e.g., methicillin resistant *Staphylococcus aureus* (MRSA), an organism that is currently sensitive to vancomycin. If MRSA acquires this resistance

mechanism, there are very few treatments left for that particularly virulent organism.

Primers were employed to amplify *vanA* and *vanB* resistance genes in Enterococci from peri-rectal swabs, and probes were employed to detect *vanA* and *vanB* resistance genes. 305 samples were tested in total, and the results compared to culture directly in samples after they were routinely processed. Using real time PCR, vancomycin resistance genes, *vanA* and *vanB*, were amplified from vancomycin resistant enterococci (VRE) directly from a peri-rectal swab. The real time PCR assay resulted in a sensitivity of 93.4% and a specificity of 99.1% (true positives 73, true negatives 224, false negatives 6, false positives 2). Thirty other lab organisms including those that reside in the gut (Figure 3) were tested with the primers and none of them were positive, thus demonstrating specificity. Therefore, such an assay can be used clinically as a diagnostic test and can yield a result the same day as the sample is obtained. Moreover, the assay is considerably more sensitive in detecting patients missed by culture.

Hence, the assay described herein overcomes the long time and low sensitivity of the current method used clinically to detect VRE. In addition, the primer and probe sets described herein to amplify and detect the *vanA* and *vanB* genes result in high sensitivity and specificity.

### Example 2

#### Cumulative Data and Use of an Internal Control

2321 specimens have been processed and tested with the assay described herein. Of those specimens, 397 (17.1%) were found to be positive. Of those positives, 312 (78.6%) contained the *vanA* gene, 73 (18.4%) the *vanB* gene, and 12 (3.0%) contained both the *vanA* and *vanB* genes.

To test for sample inhibition, an internal control was designed and incorporated into the assay. The control was plasmid DNA that contained the Ly49H gene (Figure 4), which encodes for murine natural killer cells. This DNA was added to each *vanA* reaction, with a primer and probe set (IC Forward- GCT GGC CTA AGA GTG TGT TCA GT, SEQ ID NO:19; IC Reverse- AGC CGA AGG GAA CAG AGG AT, SEQ ID NO:20; IC Probe- CCT TGG CAG CTC ATT GTG ATA GCT CTT GG, SEQ ID NO:21) designed to be specific for this

sequence, to check for sample inhibition. The internal control probe has a 3' TAMRA label. There was an approximate 1.2% inhibition rate.

To further check for specificity, many anaerobes, including gastrointestinal anaerobes which may contain the *vanB* gene, were used in the assay, such as *Eubacterium lentum*, *Clostridium innocuum* and other Clostridia species (Stinear et al., 2001; Ballard et al., 2003). Gastrointestinal anaerobes are an infection control risk. None of the additional organisms tested (Table I) for both the *vanA* and *vanB* genes were positive for either gene.

Table I

10	Eubacterium lentum
	Eubacterium sp.
	Eubacterium aerofaciens
	Clostridium innocuum
15	Clostridium difficile
	Bacteroides fragilis
	Lactobacillus sp.
	Lactococcus sp
	Prevotella sp
20	Prevotella bivia
	Fusobacterium nucleatum
	Fusobacterium sp
	Clostridium perfringens
	Clostridium glycolicum
25	Clostridium septicum
	Clostridium tertium
	Peptostreptococcus sp
	Propionabacterium sp
	Propionabacterium acnes
30	Propionabacterium granulosum

### References

- Arthur et al., J. Bacteriol., 174:2582 (1992).
- 35 Ballard et al., Abstract D-1890, 43<sup>rd</sup> ICAAC Abstract, American Society for Microbiology, p. 194 (2003).
- Barone et al., Nucl. Acids Res., 12:4051 (1984).
- Bemston et al., LPTP Newsletter, 221:1 (1998).
- Centers for Disease Control and Prevention, Morbidity and Mortality
- 40 Weekly Report, 42:597 (1993).

Centers for Disease Control and Prevention, Morbidity and Mortality Weekly Report, 44:1 (1995).

Evers et al., Microbiol Drug Resistance, 2:219 (1996).

Murray, Clin. Microbiology Rev., 3:46 (1990).

5        Persing et al, 1993. Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.

Petrich et al., Molecular and Cellular Probes, 13:275 (1999).

Petrich et al., Diag. Micro. Infect. Dis., 41:215 (2001).

Quintiliani et al., Gene, 172:1 (1996).

10        Sahun et al., J. Clin. Microbiol., 35:2026 (1997).

Sambrook et al., Molecular Cloning: A Laboratory Manual, 11 (1989).

Satake et al., J. Clin. Microbiol., 35:2325 (1997).

Stinear et al., Lancet., 357:855 (2001).

Van Horn et al., J. Clin. Microbiol., 34:924 (1996).

15

All publications, patents and patent applications are incorporated herein by reference. While in the foregoing specification, this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled  
20 in the art that the invention is susceptible to additional embodiments and that certain of the details herein may be varied considerably without departing from the basic principles of the invention.

**WHAT IS CLAIMED IS:**

1. A method to detect *vanA* in a sample, comprising:
  - a) contacting a sample suspected of comprising amplified *vanA* nucleic acid with at least one *vanA*-specific oligonucleotide probe under high stringency hybridization conditions effective to form a hybrid between the *vanA*-specific oligonucleotide probe and *vanA* nucleic acid in the sample, wherein the *vanA*-specific oligonucleotide probe comprises sequences which include sequences substantially corresponding to nucleotides 870 to 896 of the *vanA* gene, the complement thereof, or a portion thereof, sequences substantially corresponding to nucleotides 851 to 868 of the *vanA* gene, the complement thereof, or a portion thereof, or sequences substantially corresponding to nucleotides 898 to 917 of the *vanA* gene, the complement thereof, or a portion thereof; and
  - b) detecting or determining the presence or amount of hybrid formation.
2. A method to detect *vanB* in a sample, comprising:
  - a) contacting a sample suspected of comprising amplified *vanB* nucleic acid with at least one *vanB*-specific oligonucleotide probe under high stringency hybridization conditions effective to form a hybrid between the *vanB*-specific oligonucleotide probe and *vanB* nucleic acid in the sample, wherein the *vanB*-specific oligonucleotide probe comprises sequences which include sequences substantially corresponding to nucleotides 387 to 404 of the *vanB* gene, the complement thereof, or a portion thereof, sequences substantially corresponding to nucleotides 406 to 423 of the *vanB* gene, the complement thereof, or a portion thereof, or sequences substantially corresponding to nucleotides 426 to 446 of the *vanB* gene, the complement thereof, or a portion thereof; and
  - b) detecting or determining the presence or amount of hybrid formation.
3. A method to detect *vanA* in a sample, comprising:
  - a) contacting a biological sample suspected of comprising nucleic acid with at least one *vanA*-specific oligonucleotide primer under conditions effective to amplify *vanA* nucleic acid, wherein the *vanA*-specific oligonucleotide primer comprises sequences which include sequences substantially corresponding to

nucleotides 870 to 896 of the *vanA* gene, the complement thereof, or a portion thereof, sequences substantially corresponding to nucleotides 851 to 868 of the *vanA* gene, the complement thereof, or a portion thereof, or sequences substantially corresponding to nucleotides 898 to 917 of the *vanA* gene, the complement thereof, or a portion thereof; and

b) detecting or determining the presence or amount of amplified nucleic acid.

4. A method to detect *vanB* in a sample, comprising:

a) contacting a biological sample suspected of comprising nucleic acid with at least one *vanB*-specific oligonucleotide primer under conditions effective to amplify *vanB* nucleic acid, wherein the *vanB*-specific oligonucleotide primer comprises sequences which include sequences substantially corresponding to nucleotides 387 to 404 of the *vanB* gene, the complement thereof, or a portion thereof, sequences substantially corresponding to nucleotides 406 to 423 of the *vanB* gene, the complement thereof, or a portion thereof, or sequences substantially corresponding to nucleotides 426 to 446 of the *vanB* gene, the complement thereof, or a portion thereof; and

b) detecting or determining the presence or amount of amplified nucleic acid.

5. The method of claim 3 wherein one *vanA*-specific oligonucleotide primer comprises sequences corresponding to nucleotides 851 to 868 of the *vanA* gene or a portion thereof.

6. The method of claim 3 wherein one *vanA*-specific oligonucleotide primer comprises sequences corresponding to the complement of nucleotides 898 to 919 of the *vanA* gene or a portion thereof.

7. The method of claim 3 wherein the presence or amount of amplified nucleic acid is detected or determined with an oligonucleotide probe comprising sequences corresponding to nucleotides 870 to 896 of the *vanA* gene, the complement thereof or a portion thereof.

8. The method of claim 1 wherein one *vanA*-specific oligonucleotide probe comprises sequences corresponding to nucleotides 870 to 896 of the *vanA* gene, the complement thereof or a portion thereof.

9. The method of claim 8 wherein the amplified *vanB* nucleic acid is obtained by amplifying a biological sample comprising nucleic acid with at least one *vanA*-specific oligonucleotide primer comprising sequences corresponding to nucleotides 851 to 868 of the *vanA* gene or a portion thereof, or sequences corresponding to the complement of nucleotides 898 to 917 of the *vanA* gene or a portion thereof.

10. The method of claim 4 wherein one *vanB*-specific oligonucleotide primer comprises sequences corresponding to nucleotides 387 to 404 of the *vanB* gene or a portion thereof.

11. The method of claim 4 wherein one *vanB*-specific oligonucleotide primer comprises sequences corresponding to the complement of nucleotides 426 to 446 of the *vanB* gene or a portion thereof.

12. The method of claim 4 wherein the presence or amount of amplified nucleic acid is detected or determined with an oligonucleotide probe comprising sequences corresponding to nucleotides 406 to 423 of the *vanB* gene, the complement thereof or a portion thereof.

13. The method of claim 2 wherein one *vanB*-specific oligonucleotide probe comprises sequences corresponding to nucleotides 406 to 423 of the *vanB* gene, the complement thereof or a portion thereof.

14. The method of claim 13 wherein the amplified *vanB* nucleic acid is obtained by amplifying a biological sample comprising nucleic acid with at least one *vanB*-specific oligonucleotide primer comprising sequences corresponding to nucleotides 387 to 404 of the *vanB* gene or a portion thereof, or sequences corresponding to the complement of nucleotides 426 to 446 of the *vanB* gene or a portion thereof.

15. The method of claim 1, 2, 3 or 4 wherein the sample is a physiological sample.
16. The method of claim 15 wherein the sample is a peri-rectal sample.
17. The method of claim 1, 7 or 8 further comprising contacting a corresponding sample with a probe which is not a *vanA*-specific probe.
18. The method of claim 1, 7 or 8 further comprising contacting the sample with a probe which is not a *vanA*-specific probe.
19. The method of claim 17 or 18 further comprising comparing the presence or amount of hybrid formation with the *vanA*-specific oligonucleotide probe to the presence or amount of hybrid formation between the sample contacted with the non-*vanA* probe.
20. The method of claim 2, 12, or 13 further comprising contacting a corresponding sample with a probe which is not a *vanB*-specific probe.
21. The method of claim 2, 12, or 13 further comprising contacting the sample with a probe which is not a *vanB*-specific probe.
22. The method of claim 20 or 21 further comprising comparing the presence or amount of hybrid formation with the *vanB* probe to the presence or amount of hybrid formation between the sample contacted with the non-*vanB* probe.
23. The method of claim 17 or 18 wherein the non-*vanA* probe is a *vanB*-specific probe.
24. The method of claim 20 or 21 wherein the non-*vanB* probe is a *vanA*-specific probe.
25. The method of claim 7, 8, 12 or 13 wherein the probe is labeled.

26. The method of claim 23 wherein the *vanA*-specific probe is labeled with a different label than the *vanB*-specific probe.

27. The method of claim 24 wherein the *vanB*-specific probe is labeled with a different label than the *vanA*-specific probe.

28. The method of claim 18 or 21 wherein the probe which is not a *vanA*-specific probe or a *vanB*-specific probe is for an internal control.

29. A method to detect *vanA* nucleic acid and *vanB* nucleic acid in a sample, comprising:

- a) contacting a sample suspected of comprising amplified *vanA* nucleic acid or amplified *vanB* nucleic acid with at least one *vanA*-specific oligonucleotide probe and with at least one *vanB*-specific oligonucleotide probe under high stringency hybridization conditions effective to form a hybrid between the *vanA*-specific oligonucleotide probe and amplified *vanA* nucleic acid and between the *vanB*-specific oligonucleotide probe and amplified *vanB* nucleic acid, wherein the *vanA*-specific oligonucleotide probe comprises sequences which include sequences substantially corresponding to nucleotides 870 to 896 of the *vanA* gene, the complement thereof, or a portion thereof, sequences substantially corresponding to nucleotides 851 to 868 of the *vanA* gene, the complement thereof, or a portion thereof, or sequences substantially corresponding to nucleotides 898 to 917 of the *vanA* gene, the complement thereof, or a portion thereof, and wherein the *vanB*-specific oligonucleotide probe comprises sequences which include sequences substantially corresponding to nucleotides 387 to 404 of the *vanB* gene, the complement thereof, or a portion thereof, sequences substantially corresponding to nucleotides 406 to 423 of the *vanB* gene, the complement thereof, or a portion thereof, or sequences substantially corresponding to nucleotides 426 to 446 of the *vanB* gene, the complement thereof, or a portion thereof; and
- b) detecting or determining the presence or amount of hybrid formation.

30. A method to detect *vanA* nucleic acid and *vanB* nucleic acid in a sample, comprising:

- a) contacting a biological sample suspected of comprising *vanA* or *vanB* nucleic acid with at least one *vanA*-specific oligonucleotide primer under conditions effective to amplify *vanA* nucleic acid and with at least one *vanB*-specific oligonucleotide primer under conditions effective to amplify *vanB* nucleic acid, wherein the *vanA*-specific oligonucleotide primer comprises sequences which include sequences substantially corresponding to nucleotides 870 to 896 of the *vanA* gene, the complement thereof, or a portion thereof, sequences substantially corresponding to nucleotides 851 to 868 of the *vanA* gene, the complement thereof, or a portion thereof, or sequences substantially corresponding to nucleotides 898 to 917 of the *vanA* gene, the complement thereof, or a portion thereof, and wherein the *vanB*-specific oligonucleotides primer comprises sequences which include sequences substantially corresponding to nucleotides 387 to 404 of the *vanB* gene, the complement thereof, or a portion thereof, sequences substantially corresponding to nucleotides 406 to 423 of the *vanB* gene, the complement thereof, or a portion thereof, or sequences substantially corresponding to nucleotides 426 to 446 of the *vanB* gene, the complement thereof, or a portion thereof; and
- b) detecting or determining the presence or amount of amplified nucleic acid.

31. The method of claim 30 wherein the presence or amount of amplified nucleic acid is detected with at least one *vanA*-specific oligonucleotide probe and at least one *vanB*-specific oligonucleotide probe.

32. The method of claim 29 or 31 wherein the at least one *vanA*-specific oligonucleotide probe and the at least one *vanB*-specific oligonucleotide probe have different labels.

33. The method of claim 29 or 30 further comprising contacting the sample with a probe which is not a *vanA*-specific probe.

34. The method of claim 29 or 30 further comprising contacting the sample with a probe which is not a *vanB*-specific probe.

35. The method of claim 33 or 34 wherein the probe which is not a *vanA*-specific probe or a *vanB*-specific probe is for an internal control.

36. An oligonucleotide composition comprising a first oligonucleotide comprising sequences substantially corresponding to nucleotides 870 to 896 of the *vanA* gene, the complement thereof, or a portion thereof, an oligonucleotide comprising sequences substantially corresponding to nucleotides 851 to 868 of the *vanA* gene the complement thereof, or a portion thereof, an oligonucleotide comprising sequences substantially corresponding to nucleotides 898 to 917 of the *vanA* gene, the complement thereof, or a portion thereof, or a combination thereof, wherein the oligonucleotide hybridizes under stringent hybridization conditions to *vanA* DNA.

37. An oligonucleotide composition comprising an oligonucleotide comprising sequences substantially corresponding to nucleotides 387 to 404 of the *vanB* gene, the complement thereof, or a portion thereof, an oligonucleotide comprising sequences substantially corresponding to nucleotides 406 to 423 of the *vanB* gene the complement thereof, or a portion thereof, an oligonucleotide comprising sequences substantially corresponding to nucleotides 426 to 446 of the *vanB* gene, the complement thereof, or a portion thereof, or a combination thereof, wherein the oligonucleotide hybridizes under stringent hybridization conditions to *vanB* DNA.

38. The oligonucleotide composition of claim 36 wherein at least one oligonucleotide has the length and sequence of any of SEQ ID NOs:2-4.

39. The oligonucleotide composition of claim 37 wherein at least one oligonucleotide has the length and sequence of any of SEQ ID NOs:6-9.

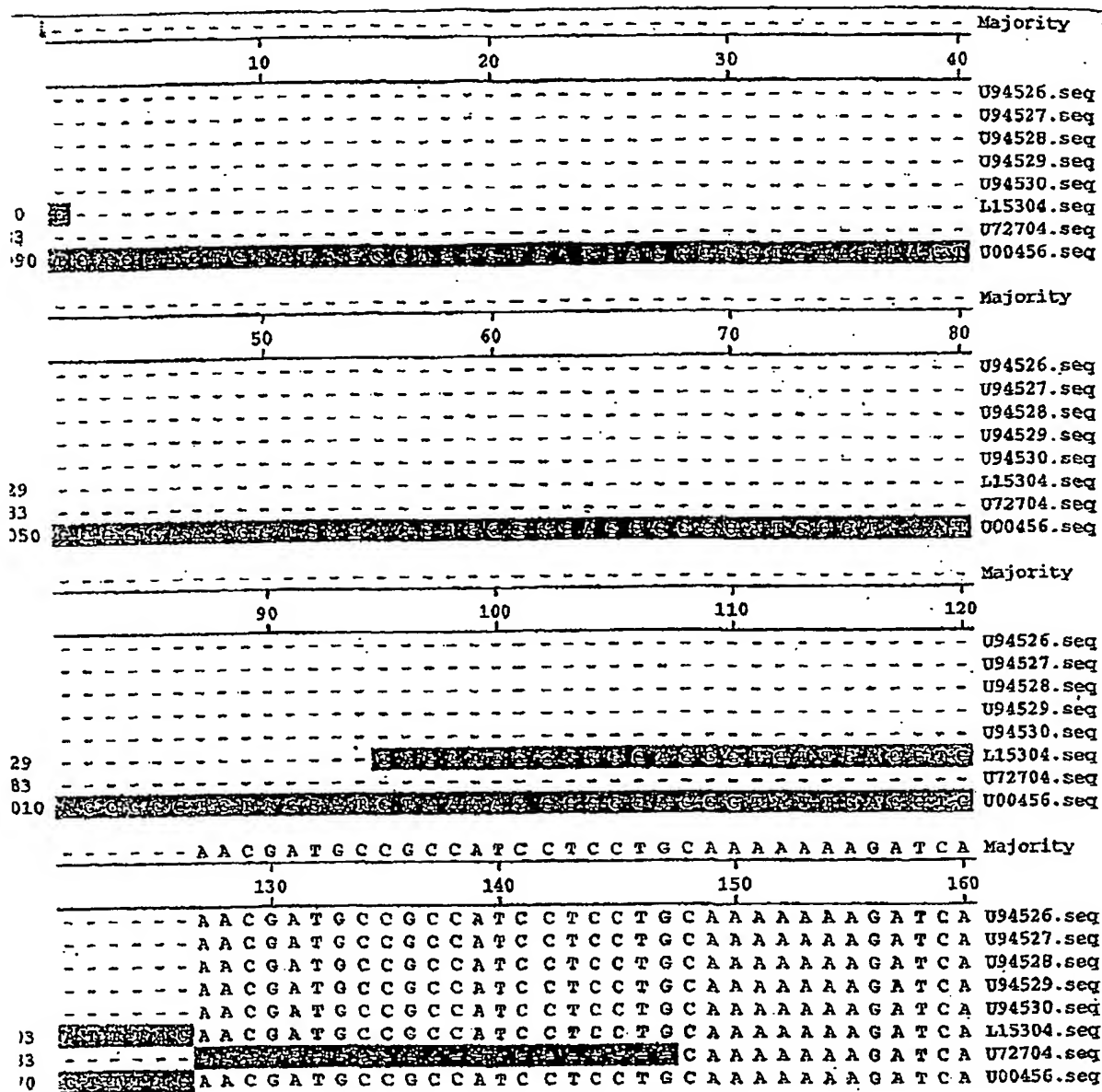
40. The oligonucleotide composition of claim 36 or 37 wherein the oligonucleotide is labeled.

41. A kit comprising an oligonucleotide specific for a *vanA* gene and/or a *vanB* gene in a test sample, comprising an oligonucleotide comprising sequences substantially corresponding to nucleotides 870 to 896 of the *vanA* gene, the complement thereof, or a portion thereof, or an oligonucleotide comprising sequences substantially corresponding to nucleotides 406 to 423 of the *vanB* gene, the complement thereof, or a portion thereof, wherein the oligonucleotide hybridizes under stringent hybridization conditions to *vanA* DNA or *vanB* DNA.
42. The kit of claim 41 further comprising at least one non-*vanA* or one non-*vanB* probe.
43. The kit of claim 41 further comprising an oligonucleotide comprising sequences substantially corresponding to nucleotides 387 to 404 of the *vanB* gene, the complement thereof, or a portion thereof, or an oligonucleotide comprising sequences substantially corresponding to nucleotides 426 to 446 of the *vanB* gene, the complement thereof, or a portion thereof.
44. The kit of claim 41 further comprising an oligonucleotide comprising sequences substantially corresponding to nucleotides 851 to 868 of the *vanA* gene, the complement thereof, or a portion thereof, or an oligonucleotide comprising sequences substantially corresponding to nucleotides 868 to 917 of the *vanA* gene, the complement thereof, or a portion thereof, or a combination thereof.
45. The kit of claim 41 wherein at least one oligonucleotide is labeled.
46. A kit comprising one or more oligonucleotides specific for a *vanA* gene in a test sample, comprising: an oligonucleotide comprising sequences substantially corresponding to nucleotides 851 to 868 of the *vanA* gene, the complement thereof, or a portion thereof, or an oligonucleotide comprising sequences substantially corresponding to nucleotides 898 to 917 of the *vanA* gene, the complement thereof, or a portion thereof, or a combination thereof.

47. A kit comprising one or more oligonucleotides specific for a *vanB* gene in a test sample, comprising: an oligonucleotide comprising sequences substantially corresponding to nucleotides 645 to 645 of the *vanB* gene, the complement thereof, or a portion thereof, or an oligonucleotide comprising sequences substantially corresponding to nucleotides 426 to 446 of the *vanB* gene, the complement thereof, or a portion thereof, or a combination thereof.

gatatcggttacgcttcatgtgccgctcaatacggatacgcactatattat  
cagccacgaacaaatacagagaatgaagcaaggagcatttcttatcaata  
ctgggcgcggtccactttagatacctatgagttggttaaagcattagaa  
aacgggaaactgggcggtgccgcatggatgtattggaaggagaggaaga  
gtttttctactctgattgcacccaaaaaccaattgataatcaatttttac  
ttaaacttcaaagaatgcctaactgataatcacaccgcatacggcctat  
tatacagagcaagcgttgctgtatccgttgaaaaaacattaaaaactg  
tttggattttgaaaggagacaggagcatgaatagaataaaagttagcaata  
ctgtttgggggttgctcagaggagcatgacgtatcggtaaaatctgcaat  
agagatagccgctaacttaataaagaaaaatacagagccgttatacattg  
gaattacgaaatctggtgtatggaaaaatgtgcgaaaaaccttgccggaa  
tggaagaaacgacaattgctattcagctgtactctcgccggataaaaaat  
gcacggattacttgttaaaaagaaacatgaatatgaaatcaaccatgttg  
atgtagcattttcagctttgcatggcaagtcaggtagaatggatccata  
caaggtctgtttgaattgtccggatccctttttaggttgcgatattca  
aagctcagcaatttgtatggacaaatcgttgacatacatcgttgcgaaaa  
atgctgggtagctactccgccttttgggttattaataaagatgatagg  
ccggtggcagctacgtttacctatcctgtttttgttaagccggcggttc  
aggtcatccctcgggtgtgaaaaaagtcaatagcgggacgaattggact  
acgcaattgaatcggcaagacaatatgacagcaaaatcttaattgagcag  
gctgtttcgggctgtgaggtcggttgtgcggtattgggaacagtgccgc  
gttagttgttggcgaggtggaccaaatcaggctgcagtacggaatctttc  
gtattcatcaggaagtcgagccggaaaaaggctctgaaaacgcagttata  
accgttccccgcagacctttcagcagaggagcaggacggatacaggaaac  
ggcaaaaaaataataaaagcgtcggctgtagaggtctagcccggtgtg  
atatgtttttacaagataacggcgcatgtactgaacgaagtcaatact  
ctgcccggtttcacgtcatacagtcgttatccccgtatgatggccgctgc  
aggtattgcacttccggaactgattgaccgcttgatcgtattagcgttaa  
aggggtgataagcatggaaataggatttacttttttagatgaaatagtac  
acgggtgttcgttgggacgctaaatatgccacttgggataatttcaccgga  
aaaccggttgacggttatgaagtaaatcgcatgtaggacatacaggtt  
ggctgaatcgcttttgaaggcaaaagaactgggtgctacccaagggtacg  
gattgcttctatgggacggttaccgtcctaagcgtgctgtaaaactgttt  
atgcaatgggctgcacagccggaaaaataacctgacaaaggaaagtatta  
tcccaatattgaccgaactgagatgatttcaaaaggatacgtggcttcaa  
aatcaagccatagccgcg

Fig 1

Fig2 (Sheet  
1 of 10)

A C A C G G G C A A G C C C T C T G C A T C C A A G C A C C C G A T A T A C T T Majority																																									
170								180								190								200																	
35	A	C	A	C	G	G	G	C	A	A	G	C	C	C	T	C	T	G	C	A	T	C	C	A	A	G	C	A	C	C	C	G	A	T	A	T	A	C	T	T	U94526.seq
35	A	C	A	C	G	G	G	C	A	A	G	C	C	C	T	C	T	G	C	A	T	C	C	A	A	G	C	A	C	C	C	G	A	T	A	T	A	C	T	T	U94527.seq
35	A	C	A	C	G	G	G	C	A	A	G	C	C	C	T	C	T	G	C	A	T	C	C	A	A	G	C	A	C	C	C	G	A	T	A	T	A	C	T	T	U94528.seq
35	A	C	A	C	G	G	G	C	A	A	G	C	C	C	T	C	T	G	C	A	T	C	C	A	A	G	C	A	C	C	C	G	A	T	A	T	A	C	T	T	U94529.seq
35	A	C	A	C	G	G	G	C	A	A	G	C	C	C	T	C	T	G	C	A	T	C	C	A	A	G	C	A	C	C	C	G	A	T	A	T	A	C	T	T	U94530.seq
563	A	C	A	C	G	G	G	C	A	A	G	C	C	C	T	C	T	G	C	A	T	C	C	A	A	G	C	A	C	C	C	G	A	T	A	T	A	C	T	T	L15304.seq
770	A	C	A	C	G	G	G	C	A	A	G	C	C	C	T	C	T	G	C	A	T	C	C	A	A	G	C	A	C	C	C	G	A	T	A	T	A	C	T	T	U72704.seq
930	A	C	A	C	G	G	G	C	A	A	G	C	C	C	T	C	T	G	C	A	T	C	C	A	A	G	C	A	C	C	C	G	A	T	A	T	A	C	T	T	U00456.seq
T C T T T G C C G T T T C C T G C A C C C G A T T T C G T T C C T C G A C C G G Majority																																									
210								220								230								240																	
75	T	C	T	T	T	G	C	C	G	T	T	T	C	C	T	G	C	A	C	C	C	G	A	T	T	T	C	G	T	T	C	C	T	C	G	A	C	C	G	G	U94526.seq
75	T	C	T	T	T	G	C	C	G	T	T	T	C	C	T	G	C	A	C	C	C	G	A	T	T	T	C	G	T	T	C	C	T	C	G	A	C	C	G	G	U94527.seq
75	T	C	T	T	T	G	C	C	G	T	T	T	C	C	T	G	C	A	C	C	C	G	A	T	T	T	C	G	T	T	C	C	T	C	G	A	C	C	G	G	U94528.seq
75	T	C	T	T	T	G	C	C	G	T	T	T	C	C	T	G	C	A	C	C	C	G	A	T	T	T	C	G	T	T	C	C	T	C	G	A	C	C	G	G	U94529.seq
75	T	C	T	T	T	G	C	C	G	T	T	T	C	C	T	G	C	A	C	C	C	G	A	T	T	T	C	G	T	T	C	C	T	C	G	A	C	C	G	G	U94530.seq
523	T	C	T	T	T	G	C	C	G	T	T	T	C	C	T	G	C	A	C	C	C	G	A	T	T	T	C	G	T	T	C	C	T	C	G	A	C	C	G	G	L15304.seq
730	T	C	T	T	T	G	C	C	G	T	T	T	C	C	T	G	C	A	C	C	C	G	A	T	T	T	C	G	T	T	C	C	T	C	G	A	C	C	G	G	U72704.seq
890	T	C	T	T	T	G	C	C	G	T	T	T	C	C	T	G	C	A	C	C	C	G	A	T	T	T	C	G	T	T	C	C	T	C	G	A	C	C	G	G	U00456.seq
A A T G T C T G C G G G A A C T G T A A T C A T C G C A T T T T C T G A G C C T Majority																																									
250								260								270								280																	
115	A	A	T	G	T	C	T	G	C	G	G	G	A	A	C	T	G	T	A	A	T	C	A	T	C	G	C	A	T	T	T	T	C	T	G	A	G	C	C	T	U94526.seq
115	A	A	T	G	T	C	T	G	C	G	G	G	A	A	C	T	G	T	A	A	T	C	A	T	C	G	C	A	T	T	T	T	C	T	G	A	G	C	C	T	U94527.seq
115	A	A	T	G	T	C	T	G	C	G	G	G	A	A	C	T	G	T	A	A	T	C	A	T	C	G	C	A	T	T	T	T	C	T	G	A	G	C	C	T	U94528.seq
115	A	A	T	G	T	C	T	G	C	G	G	G	A	A	C	T	G	T	A	A	T	C	A	T	C	G	C	A	T	T	T	T	C	T	G	A	G	C	C	T	U94529.seq
115	A	A	T	G	T	C	T	G	C	G	G	G	A	A	C	T	G	T	A	A	T	C	A	T	C	G	C	A	T	T	T	T	C	T	G	A	G	C	C	T	U94530.seq
483	A	A	T	G	T	C	T	G	C	G	G	G	A	A	C	T	G	T	A	A	T	C	A	T	C	G	C	A	T	T	T	T	C	T	G	A	G	C	C	T	L15304.seq
690	A	A	T	G	T	C	T	G	C	G	G	G	A	A	C	T	G	T	A	A	T	C	A	T	C	G	C	A	T	T	T	T	C	T	G	A	G	C	C	T	U72704.seq
850	A	A	T	G	T	C	T	G	C	G	G	G	A	A	C	T	G	T	A	A	T	C	A	T	C	G	C	A	T	T	T	T	C	T	G	A	G	C	C	T	U00456.seq
T T T T C C G G C T C G T T T T C C T G A T G G A T G C G G A A G A T A C C G T Majority																																									
290								300								310								320																	
155	T	T	T	T	C	C	G	G	C	T	C	G	T	T	T	T	C	T	G	A	T	G	G	A	T	G	C	G	G	A	A	G	A	T	A	C	C	G	T	U94526.seq	
155	T	T	T	T	C	C	G	G	C	T	C	G	T	T	T	T	C	C	T	G	A	T	G	G	A	T	G	C	G	G	A	A	G	A	T	A	C	C	G	T	U94527.seq
155	T	T	T	T	C	C	G	G	C	T	C	G	T	T	T	T	C	C	T	G	A	T	G	G	A	T	G	C	G	G	A	A	G	A	T	A	C	C	G	T	U94528.seq
155	T	T	T	T	C	C	G	G	C	T	C	G	T	T	T	T	C	C	T	G	A	T	G	G	A	T	G	C	G	G	A	A	G	A	T	A	C	C	G	T	U94529.seq
155	T	T	T	T	C	C	G	G	C	T	C	G	T	T	T	T	C	C	T	G	A	T	G	G	A	T	G	C	G	G	A	A	G	A	T	A	C	C	G	T	U94530.seq
443	T	T	T	T	C	C	G	G	C	T	C	G	T	T	T	T	C	C	T	G	A	T	G	G	A	T	G	C	G	G	A	A	G	A	T	A	C	C	G	T	L15304.seq
650	T	T	T	T	C	C	G	G	C	T	C	G	T	T	T	T	C	C	T	G	A	T	G	G	A	T	G	C	G	G	A	A	G	A	T	A	C	C	G	T	U72704.seq
810	T	T	T	T	C	C	G	G	C	T	C	G	T	T	T	T	C	C	T	G	A	T	G	G	A	T	G	C	G	G	A	A	G	A	T	A	C	C	G	T	U00456.seq

FIG. 2 (sheet 2 of 10)  
cont'd

	GGCTCAGCCCGGATTGTGATCCACTTCGCCGACCAATCAAAATC Majority																												
	330							340							350							360							
195	GGCTCAGCCCGGATTGTGATCCACTTCGCCGACCAATCAAAATC	U94526.seq																											
195	GGCTCAGCCCGGATTGTGATCCACTTCGCCGACCAATCAAAATC	U94527.seq																											
195	GGCTCAGCCCGGATTGTGATCCACTTCGCCGACCAATCAAAATC	U94528.seq																											
195	GGCTCAGCCCGGATTGTGATCCACTTCGCCGACCAATCAAAATC	U94529.seq																											
195	GGCTCAGCCCGGATTGTGATCCACTTCGCCGACCAATCAAAATC	U94530.seq																											
403	GGCTCAGCCCGGATTGTGATCCACTTCGCCGACCAATCAAAATC	L15304.seq																											
610	GGCTCAGCCCGGATTGTGATCCACTTCGCCGACCAATCAAAATC	U72704.seq																											
770	GGCTCAGCCCGGATTGTGATCCACTTCGCCGACCAATCAAAATC	U00456.seq																											
ATCCTCGTTTCCCCATGACCGCACACCCGACCTCACAGCCC Majority																													
	370							380							390							400							
235	ATCCTCGTTTCCCCATGACCGCACACCCGACCTCACAGCCC	U94526.seq																											
235	ATCCTCGTTTCCCCATGACCGCACACCCGACCTCACAGCCC	U94527.seq																											
235	ATCCTCGTTTCCCCATGACCGCACACCCGACCTCACAGCCC	U94528.seq																											
235	ATCCTCGTTTCCCCATGACCGCACACCCGACCTCACAGCCC	U94529.seq																											
235	ATCCTCGTTTCCCCATGACCGCACACCCGACCTCACAGCCC	U94530.seq																											
363	ATCCTCGTTTCCCCATGACCGCACACCCGACCTCACAGCCC	L15304.seq																											
570	ATCCTCGTTTCCCCATGACCGCACACCCGACCTCACAGCCC	U72704.seq																											
730	ATCCTCGTTTCCCCATGACCGCACACCCGACCTCACAGCCC	U00456.seq																											
GAAATCGCTTGCTCAATTAAAGATTTTTCCATCATATTGTTC Majority																													
	410							420							430							440							
275	GAAATCGCTTGCTCAATTAAAGATTTTTCCATCATATTGTTC	U94526.seq																											
275	GAAATCGCTTGCTCAATTAAAGATTTTTCCATCATATTGTTC	U94527.seq																											
275	GAAATCGCTTGCTCAATTAAAGATTTTTCCATCATATTGTTC	U94528.seq																											
275	GAAATCGCTTGCTCAATTAAAGATTTTTCCATCATATTGTTC	U94529.seq																											
275	GAAATCGCTTGCTCAATTAAAGATTTTTCCATCATATTGTTC	U94530.seq																											
323	GAAATCGCTTGCTCAATTAAAGATTTTTCCATCATATTGTTC	L15304.seq																											
530	GAAATCGCTTGCTCAATTAAAGATTTTTCCATCATATTGTTC	U72704.seq																											
690	GAAATCGCTTGCTCAATTAAAGATTTTTCCATCATATTGTTC	U00456.seq																											
CTGCCGCTTCTATCGCAGCGTTAAAGTTCTTCCGTACCGTTT Majority																													
	450							460							470							480							
315	CTGCCGCTTCTATCGCAGCGTTAAAGTTCTTCCGTACCGTTT	U94526.seq																											
315	CTGCCGCTTCTATCGCAGCGTTAAAGTTCTTCCGTACCGTTT	U94527.seq																											
315	CTGCCGCTTCTATCGCAGCGTTAAAGTTCTTCCGTACCGTTT	U94528.seq																											
315	CTGCCGCTTCTATCGCAGCGTTAAAGTTCTTCCGTACCGTTT	U94529.seq																											
315	CTGCCGCTTCTATCGCAGCGTTAAAGTTCTTCCGTACCGTTT	U94530.seq																											
283	CTGCCGCTTCTATCGCAGCGTTAAAGTTCTTCCGTACCGTTT	L15304.seq																											
490	CTGCCGCTTCTATCGCAGCGTTAAAGTTCTTCCGTACCGTTT	U72704.seq																											
650	CTGCCGCTTCTATCGCAGCGTTAAAGTTCTTCCGTACCGTTT	U00456.seq																											

FIG. 2 (continued)  
Sheet 3 of 10

T A C T T T G G T T A C G C C A A A G G A C G A A C C T G A C C G T G C C G G C																																Majority												
490								500								510								520																				
355	T	A	C	T	T	T	G	G	T	T	A	C	G	C	C	A	A	A	G	G	A	C	G	A	A	C	C	T	G	A	C	C	G	T	G	C	C	G	G	C	U94526.seq			
355	T	A	C	T	T	T	G	G	T	T	A	C	G	C	C	A	A	A	G	G	A	C	G	A	A	C	C	T	G	A	C	C	G	T	G	C	C	G	G	C	U94527.seq			
355	T	A	C	T	T	T	G	G	T	T	A	C	G	C	C	A	A	A	G	G	A	C	G	A	A	C	C	T	G	A	C	C	G	T	G	C	C	G	G	C	U94528.seq			
355	T	A	C	T	T	T	G	G	T	T	A	C	G	C	C	A	A	A	G	G	A	C	G	A	A	C	C	T	G	A	C	C	G	T	G	C	C	G	G	C	U94529.seq			
355	T	A	C	T	T	T	G	G	T	T	A	C	G	C	C	A	A	A	G	G	A	C	G	A	A	C	C	T	G	A	C	C	G	T	G	C	C	G	G	C	U94530.seq			
243	T	A	C	T	T	T	G	G	T	T	A	C	G	C	C	A	A	A	G	G	A	C	G	A	A	C	C	T	G	A	C	C	G	T	G	C	C	G	G	C	L15304.seq			
450	T	A	C	T	T	T	G	G	T	T	A	C	G	C	C	A	A	A	G	G	A	C	G	A	A	C	C	T	G	A	C	C	G	T	G	C	C	G	G	C	U72704.seq			
610	T	A	C	T	T	T	G	G	T	T	A	C	G	C	C	A	A	A	G	G	A	C	G	A	A	C	C	T	G	A	C	C	G	T	G	C	C	G	G	C	U00456.seq			
T T C A C A A A G A C A G G G T A G G T A A G C G C A C C C G C C T C C G G C T																																Majority												
530								540								550								560																				
395	T	T	C	A	C	A	A	A	G	A	C	A	G	G	G	T	A	G	G	T	A	A	G	C	G	C	A	C	C	C	G	C	C	T	C	C	G	G	C	T	U94526.seq			
395	T	T	C	A	C	A	A	A	G	A	C	A	G	G	G	T	A	G	G	T	A	A	G	C	G	C	A	C	C	C	G	C	C	T	C	C	G	G	C	T	U94527.seq			
395	T	T	C	A	C	A	A	A	G	A	C	A	G	G	G	T	A	G	G	T	A	A	G	C	G	C	A	C	C	C	G	C	C	T	C	C	G	G	C	T	U94528.seq			
395	T	T	C	A	C	A	A	A	G	A	C	A	G	G	G	T	A	G	G	T	A	A	G	C	G	C	A	C	C	C	G	C	C	T	C	C	G	G	C	T	U94529.seq			
395	T	T	C	A	C	A	A	A	G	A	C	A	G	G	G	T	A	G	G	T	A	A	G	C	G	C	A	C	C	C	G	C	C	T	C	C	G	G	C	T	U94530.seq			
203	T	T	C	A	C	A	A	A	G	A	C	A	G	G	G	T	A	G	G	T	A	A	G	C	G	C	A	C	C	C	G	C	C	T	C	C	G	G	C	T	L15304.seq			
410	T	T	C	A	C	A	A	A	G	A	C	A	G	G	G	T	A	G	G	T	A	A	G	C	G	C	A	C	C	C	G	C	C	T	C	C	G	G	C	T	U72704.seq			
570	T	T	C	A	C	A	A	A	G	A	C	A	G	G	G	T	A	G	G	T	A	A	G	C	G	C	A	C	C	C	G	C	C	T	C	C	G	G	C	T	U00456.seq			
T G T C A C C T T T T A T C A A T C A T T T G A A A T T C G G G A A C G G C G A T																																Majority												
570								580								590								600																				
435	T	G	T	C	A	C	C	T	T	T	A	T	C	A	A	T	C	A	T	T	T	G	A	A	A	T	T	C	G	G	G	A	A	C	G	G	C	G	A	T	U94526.seq			
435	T	G	T	C	A	C	C	T	T	T	T	C	A	A	T	C	A	T	T	T	T	G	A	A	A	T	T	C	G	G	G	A	A	C	G	G	C	G	A	T	U94527.seq			
435	T	G	T	C	A	C	C	T	T	T	A	T	C	A	A	T	C	A	T	T	T	G	A	A	A	T	T	C	G	G	G	A	A	C	G	G	C	G	A	T	U94528.seq			
435	T	G	T	C	A	C	C	T	T	T	A	T	C	A	A	T	C	A	T	T	T	G	A	A	A	T	T	C	G	G	G	A	A	C	G	G	C	G	A	T	U94529.seq			
435	T	G	T	C	A	C	C	T	T	T	A	T	C	A	A	T	C	A	T	T	T	G	A	A	A	T	T	C	G	G	G	A	A	C	G	G	C	G	A	T	U94530.seq			
163	T	G	T	C	A	C	C	T	T	T	A	T	C	A	A	T	C	A	T	T	T	G	A	A	A	T	T	C	G	G	G	A	A	C	G	G	C	G	A	T	L15304.seq			
370	T	G	T	C	A	C	C	T	T	T	A	T	C	A	A	T	C	A	T	T	T	G	A	A	A	T	T	C	G	G	G	A	A	C	G	G	C	G	A	T	U72704.seq			
530	T	G	T	C	A	C	C	T	T	T	T	C	A	A	T	C	A	T	T	T	T	G	A	A	A	T	T	C	G	G	G	A	A	C	G	G	C	G	A	T	U00456.seq			
G C C C G C A T T T T T T G T A A G A A T G T A G G C C A G T G A T T T G T C C																																Majority												
610								620								630								640																				
175	G	C	C	C	G	C	A	T	T	T	T	T	T	T	T	G	T	A	A	G	A	A	T	G	T	A	G	G	C	C	A	G	T	G	A	T	T	T	G	T	C	U94526.seq		
175	G	C	C	C	G	C	A	T	T	T	T	T	T	T	T	T	G	T	A	A	G	A	A	T	G	T	A	G	G	C	C	A	G	T	G	A	T	T	T	G	T	C	U94527.seq	
175	G	C	C	C	G	C	A	T	T	T	T	T	T	T	T	T	G	T	A	A	G	A	A	T	G	T	A	G	G	C	C	A	G	T	G	A	T	T	T	G	T	C	U94528.seq	
175	G	C	C	C	G	C	A	T	T	T	T	T	T	T	T	T	G	T	A	A	G	A	A	T	G	T	A	G	G	C	C	A	G	T	G	A	T	T	T	G	T	C	U94529.seq	
175	G	C	C	C	G	C	A	T	T	T	T	T	T	T	T	T	G	T	A	A	G	A	A	T	G	T	A	G	G	C	C	A	G	T	G	A	T	T	T	G	T	C	U94530.seq	
123	G	C	C	C	G	C	A	T	T	T	T	T	T	T	T	T	T	G	T	A	A	G	A	A	T	G	T	A	G	G	C	C	A	G	T	G	A	T	T	T	G	T	C	L15304.seq
330	G	C	C	C	G	C	A	T	T	T	T	T	T	T	T	T	T	G	T	A	A	G	A	A	T	G	T	A	G	G	C	C	A	G	T	G	A	T	T	T	G	T	C	U72704.seq
190	G	C	C	C	G	C	A	T	T	T	T	T	T	T	T	T	T	G	T	A	A	G	A	A	T	G	T	A	G	G	C	C	A	G	T	G	A	T	T	T	G	T	C	U00456.seq

FIG. 2 (cont'd)  
Sheet 4 of 10



	GCCCATGCGTTTTCCTATCCGGGGAGAGTATGGCGGGGAG																																Majority								
	810								820								830								840																
675	G	C	C	C	A	T	G	C	G	T	T	T	T	C	C	T	A	T	C	C	G	G	G	G	A	G	A	G	T	A	T	G	G	C	G	G	G	G	A	G	U94526.seq
675	G	C	C	C	A	T	G	C	G	T	T	T	T	C	C	T	A	T	C	C	G	G	G	G	A	G	A	T	A	T	G	G	C	G	G	G	G	A	G	U94527.seq	
675	G	C	C	C	A	T	G	C	G	T	T	T	T	C	C	T	A	T	C	C	G	G	G	A	G	A	G	T	A	T	G	G	C	G	G	G	A	G	U94528.seq		
675	G	C	C	C	A	T	G	C	G	T	T	T	T	C	C	T	A	T	C	C	G	G	G	A	G	A	G	T	A	T	G	G	C	G	G	G	A	G	U94529.seq		
675	G	C	C	C	A	T	G	C	G	T	T	T	T	C	C	T	A	T	C	C	G	G	G	A	G	A	G	T	A	T	G	G	C	G	G	G	A	G	U94530.seq		
4	[REDACTED]																																L15304.seq								
130	G	C	C	A	T	G	C	G	T	T	T	T	T	C	C	T	A	T	C	C	G	G	G	A	G	A	G	T	A	T	G	G	C	G	G	G	A	G	U72704.seq		
290	G	C	C	A	T	G	C	G	T	T	T	T	T	C	C	T	A	T	C	C	G	G	G	A	G	A	T	A	T	G	G	C	G	G	G	A	G	U00456.seq			
																																	Majority								
	ACTGTCGGCTTTC CCA TTCCGTACATGGCTTCTTG CATAGC																																								
	850								860								870								880																
715	A	C	T	G	T	C	G	G	C	T	T	C	C	C	A	T	T	C	C	G	T	A	C	A	T	G	G	C	T	T	C	T	T	G	C	A	T	A	G	C	U94526.seq
715	A	C	T	G	T	C	G	G	C	T	T	C	C	C	A	T	T	C	C	G	T	A	C	A	T	G	G	C	T	T	C	T	T	G	C	A	T	A	G	C	U94527.seq
715	A	C	T	G	T	C	G	G	C	T	T	C	C	C	A	T	T	C	C	G	T	A	C	A	T	G	G	C	T	T	C	T	T	G	C	A	T	A	G	C	U94528.seq
715	A	C	T	G	T	C	G	G	C	T	T	C	C	C	A	T	T	C	C	G	T	A	C	A	T	G	G	C	T	T	C	T	T	G	C	A	T	A	G	C	U94529.seq
715	A	C	T	G	T	C	G	G	C	T	T	C	C	C	A	T	T	C	C	G	T	A	C	A	T	G	G	C	T	T	C	T	T	G	C	A	T	A	G	C	U94530.seq
4	[REDACTED]																																L15304.seq								
90	A	C	T	G	T	C	G	G	C	T	T	C	C	C	A	T	T	C	C	G	T	A	C	A	T	G	G	C	T	T	C	T	T	G	C	A	T	A	G	C	U72704.seq
250	A	C	T	G	T	C	G	G	C	T	T	C	C	C	A	T	T	C	C	G	T	A	C	A	T	G	G	C	T	T	C	T	T	G	C	A	T	A	G	C	U00456.seq
																																	Majority								
	T T C C A T A C A C C G T T T T T T G T A A T T C C G A T G T A G T G C G G A T																																								
	890								900								910								920																
755	T	T	C	C	A	T	A	C	A	C	C	G	T	T	T	T	T	T	G	T	A	A	T	T	C	C	G	A	T	G	T	A	G	T	G	C	G	G	A	T	U94526.seq
755	T	T	C	C	A	T	A	C	A	C	C	G	T	T	T	T	T	T	G	T	A	A	T	T	C	C	G	A	T	G	T	A	G	T	G	C	G	G			

FIG. 2 (cont'd)  
Sheet 6 of 10

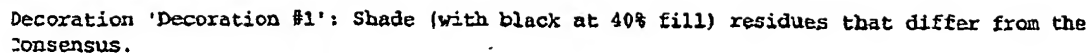


FIG. 2 (cont'd)  
Sheet 7 of 10

```

1 aacgatgccg ccatectcct gcaaaaaaag atcaaacacgg gcaagccctc tgcateccaag
61 cacccgatat actttctttg ccgtttcctg cacccgattt cgttcctcga ccggaatgtc
121 tgccgggaact gtaatcatcg cattttctga gcctttttcc ggctcgtttt cctgatggat
181 gcggaagata ccgtggctca gccggatttg atccacttcg ccgacaatca aatcatcctc
241 gttccccatg accgcacacc cgacctcaca gcccgaaatc gcttgctcaa ttaagatttt
301 tccatcatat tgcctgccc cttctatcgc agcgtaaagt tcttcctgac cgtttacttt
361 ggttacgcca aaggacgaac ctgaccgtgc cggcttcaca aagacagggg aggtaaagcg
421 acccgccctcc ggcttgctac ctttatcaat catttgaaat tcgggaacgg cgatgcccgc
481 attttttgta agaattgtagg ccagtgattt gtccatgcaa gctgcggagc tttgaatata
541 acagcccaca taggggatac cagacaatac aaacagcccc tgtatcgcac catcctcccc
601 gcatttgcca tgcaaaaccg ggaaagccac atcaatacgc cgtgtttcgt attcgttttc
661 tttcatgaca agcagcccat gcgttttctt atccggggag agtatggcgg ggagactgtc
721 ggcttcccat tccgtacatg gcttcttgca tagcttccat acaccgtttt ttgtaattcc
781 gatgtagtgc ggatcgaatt t

```

SEQ ID NO: 10

```

1 aacgatgccg ccatectcct gcaaaaaaag atcaaacacga gcaagccctc tgcateccaag
61 cacccgatat actttctttg ccgtttcctg cacccgattt cgttcctcga ccggaatgtc
121 tgctggaaag ataatcatcg cattttctga gcctttttcc ggctcgtttt cctgatggat
181 gcggaagata ccgtggctca accggatttg atccacttcg ccgacaatca aatcatcctc
241 gtttcccatg accgcgcagc cgacctcaca gcccgaaatc gcttgctcaa ttaagatttt
301 tccatcatat tgcctgctg cttctatcgc agcgtttagt tcttcctgac tgtttacttt
361 ggttacgcca aaggacgaac ctgaccgtgc cggcttcaca aagacagggg aggtaaagcg
421 cctcgccctcc ggtttgctac ctttttcaat catttgaaat tcggggagcg cgatgcccgc
481 attttttgta agaattgtagg ccagtgattt gtccatgcaa gctgcggagc tttgaatata
541 gcagccctaca taggggatac cagacaattc aaacagaccg tgtatcgcac catcctcccc
601 gcatttgcca tgcaaaaccg ggaaagccac gtcaatacgc cgagtttcgt attctctttc
661 tttcatgaca agcagcccat gcgttttctt atccggggag aatatggcgg ggagactgtc
721 ggcttcccat tccgtacatg gcttcttgca tagcttccat acgcggtttt ttgtaattcc
781 gatgtagtgc ggatcgaatt t

```

SEQ ID NO: 11

```

1 aacgatgccg ccatectcct gcaaaaaaag atcaaacacgg gcaagccctc tgcateccaag
61 cacccgatat actttctttg ccgtttcctg cacccgattt cgttcctcga ccggaatgtc
121 tgccgggaact gtaatcatcg cattttctga gcctttttcc ggctcgtttt cctgatggat
181 gcggaagata ccgtggctca gccggatttg atccacttcg ccgacaatca aatcatcctc
241 gttccccatg accgcacacc cgacctcaca gcccgaaatc gcttgctcaa ttaagatttt
301 tccatcatat tgcctgccc cttctatcgc agcgtaaagt tcttcctgac cgtttacttt
361 ggttacgcca aaggacgaac ctgaccgtgc cggcttcaca aagacagggg aggtaaagcg
421 acccgccctcc ggcttgctac ctttatcaat aatttgaaat tcgggaacgg cgatgcccgc
481 attttttgta agaattgtagg ccagtgattt gtccatgcaa gctgcggagc tttgaatata
541 acagcccaca taggggatac cagacaatac aaacagcccc tgtatcgcac catcctcccc
601 gcatttgcca tgcaaaaccg ggaaagccac atcaatacgc cgtgtttcgt attcgttttc
661 tttcatgaca agcagcccat gcgttttctt atccggggag agtatggcgg ggagactgtc
721 ggcttcccat tccgtacatg gcttcttgca tagcttccat acaccgtttt ttgtaattcc
781 gatgtagtgc ggatcgaatt t

```

SEQ ID NO: 12

FIG. 2 (cont'd)  
Sheet 8 of 10

```

1 aacgatgccg ccatectcct gcaaaaaaag atcaacacgg gcaagccctc tgcattccaag
61 cacccgatat actttctttg ccgtttcctg cacccgattt cgttcctcga ccggaatgtc
121 tgcgggaact gtaatcatcg cattttctga gcctttttcc ggctcgtttt cctgatggat
181 gcggaagata ccgtggctca gccggatttg atccacttcg ccgacaatca aatcatcctc
241 gttccccatg accgcacacc cgacctcaca gcccgaaatc gcttgctcaa ttaagatttt
301 tccatcatat tgcctgcccg cttctatcgc agcgttaagt tcttcctgac cgtttacttt
361 ggttacgcca aaggacgaac ctgaccgtgc cggcttcaca aagacagggg aggttaagcgc
421 acccgctccc ggcttgctac ctttatcaat aatttgaaat tcgggaacgg cgtatgccgc
481 attttttgta agaattgtagg ccagtgtatt gtccatgcaa gctgcggagc tttgaatatc
541 acagcccaca taggggatac cagacaatac aaacagcccc tgtatcgcac catcctcccc
601 gcatttgcca tgcaaaaccg ggaagaccac atcaatacgc cgtgtttcgt attcgttttc
661 tttcatgaca agcagcccat gcgttttcct atccggggag agtatggcgg ggagactgtc
721 ggcttcccat tccgtacatg gcttcttgca tagcttccat acaccgtttt ttgtaattcc
781 gatgtagtgc ggaatgaatt t

```

SEQ ID NO: 13

```

1 aacgatgccg ccatectcct gcaaaaaaag atcaacacgg gcaagccctc tgcattccaag
61 cacccgatat actttctttg ccgtttcctg cacccgattt cgttcctcga ccggaatgtc
121 tgcgggaact gtaatcatcg cattttctga gcctttttcc ggctcgtttt cctgatggat
181 gcggaagata ccgtggctca gccggatttg atccacttcg ccgacaatca aatcatcctc
241 gttccccatg accgcacacc cgacctcaca gcccgaaatc gcttgctcaa ttaagatttt
301 tccatcatat tgcctgcccg cttctatcgc agcgttaagt tcttcctgac cgtttacttt
361 ggttacgcca aaggacgaac ctgaccgtgc cggcttcaca aagacagggg aggttaagcgc
421 acccgctccc ggcttgctac ctttatcaat aatttgaaat tcgggaacgg cgtatgccgc
481 attttttgta agaattgtagg ccagtgtatt gtccatgcaa gctgcggagc tttgaatatc
541 acagcccaca taggggatac cagacaatac aaacagcccc tgtatcgcac catcctcccc
601 gcatttgcca tgcaaaaccg ggaagaccac atcaatacgc cgtgtttcgt attcgttttc
661 tttcatgaca agcagcccat gcgttttcct atccggggag agtatggcgg ggagactgtc
721 ggcttcccat tccgtacatg gcttcttgca tagcttccat acaccgtttt ttgtaattcc
781 gatgtagtgc ggaatgaatt t

```

SEQ ID NO: 14

```

1 gaggatgggt gcatccaggg actgtttgaa ttgtctggta tccctatgt gggctgtgat
61 attcaaagct ccgcagcttg catggacaaa tcactggcct acattcttac aaaaaatgoc
121 ggcacgcccg ttcccgaaat tcaaatgatt gataaagggt acaagccgga ggcgggtgoc
181 cttacctacc ctgtctttgt gaagccggca cgtcagggtt cgtcctttgg cgttaaccaa
241 gtaaacggta cgaagaact taacgtgctg atagaagcgg caggacaata tga tggaaaa
301 atcttaattg agcaagcgat ttccggctgt gaggtcgggt gtgcggctcat ggggaacgag
361 gatgatttga ttgtcggcga agtgatcaa atccggctga gccacgggtat cttccgcac
421 catcaggaaa acgagccgga aaaaggctca gaaaatgca tgattacagt tcccgagac
481 attccggtcg aggaacgaaa tcgggtgcag gaaacggcaa agaaagtata tcgggtgctt
541 ggatgcagag ggcttgcccg tgttgatctt tttttgcagg aggatggcgg catcgttcta
601 aatgaggtca acaccctgcc cggcttcacg

```

SEQ ID NO: 15

FIG. 2 (cont'd)  
Sheet 9 of 10

```

1 gaaaaattcg atccgcacta catcggaatt acaaaaagggt gtgtatggaa gctatgcaag
61 aagccatgta cgggaatggga agccgacagt ctcccgcgca tactctcccc ggataggaaa
121 acgcatggtc tgcttgtcat gaaagaaagc gaatacgaac cacggcggtat tgatgtggct
181 ttcccagttt tgcattggcaa atgcggggag gacggtgcga tacagggttt atttgaattg
241 tctggcatcc cctatgtggg ctgcgatatt caaagctccg cagcttgcat ggacaaatca
301 ctggcctaca ttcttacaaa aaatgcgggc atcgccgttc ccgaatttca aatgattgat
361 aaaggtgaca agccggagac ggggtgcgctt acctaccctg tctttgtgaa gccggcacgg
421 tcaggttcgt cctttggctt aaccaaagta aacggtacgg aagaacttaa cgctgcgata
481 gaagcggcag gacaatatga tggaaaaatc ttaattgagc aagcgatttc gggctgtgag
541 gtcggctgtg cggttatggg gaacgaggat gatttgattg tcggcgaaat ggatcaaatc
601 cggctgagcc atggtatctt ccgcattcat caggaaaacg agccggaaaa aggatcagag
661 aatgcgatga ttaccgttcc tgcagacatc ccagtcgggg aacgaaatcg ggtgcaggaa
721 acggcacaaga aagtatatcg ggcgcttggg tgcagagggc ttgcccggtg tgatcttttt
781 ttg

```

SEQ ID NO: 16

```

1 tgctgcgaga taccacagaa aacaatcagg aattgtctta actttgaaag gagtttacag
61 catgaataaa ataaaagtcg caattatctt cggcggttgc tcggaggaac atgatgtgc
121 ggtaaaatcc gcaatagaaa ttgctgcgaa cattaatact gaaaaattcg atccgcacta
181 catcggaatt acaaaaaacg gcgtatggaa gctatgcaag aagccatgta cgggaatggga
241 agccgatagt ctcccgcgca tattctcccc ggataggaaa acgcatggtc tgcttgcatt
301 gaaagaaaga gaatacgaac ctcgccgtat tgacgtggct ttcccgggtt tgcattggca
361 atgcggggag gatgggtcga tacagggtct gtttgaattg tctgggtatc cctatgtagg
421 ctgcgatatt caaagctccg cagcttgcat ggacaaatca ctggcctaca ttcttacaaa
481 aaatgcgggc atcgccgtcc ccgaatttca aatgattgaa aaaggtgaca aaccggaggg
541 gaggacgctt acctaccctg tctttgtgaa gccggcacgg tcaggttcgt cctttggcgt
601 aaccaaagta aacagtacgg aagaactaaa cgctgcgata gaagcagcag gacaatatga
661 tggaaaaatc ttaattgagc aagcgatttc gggctgtgag gtcggctgcg cggctcatggg
721 aaacgaggat gatttgattg tcggcgaaat ggatcaaatc cggttgagcc acggtatctt
781 ccgcattcat caggaaaaac agccggaaaa aggcacagag aatgcgatga ttatcgttcc
841 agcagacatt ccggtcgagg aacgaaatcg ggtgcaagaa acggcacaaga aagtatatcg
901 ggtgcttggg tgcagagggc ttgctcgtgt tgatcttttt ttgcaggagg atggcggcat
961 cgttctaaac gaggtcaata ccctgcccg ttttacatcg tacagccgct atccacgcat
1021 ggcggctgcc gcaggaaatc cgcttcccgc actaattgac agcctgatta cattggcgat
1081 agagaggtga

```

SEQ ID NO: 5

FIG. 2 (cont'd)  
Sheet 10 of 10

Escherichia coli  
Klebsiella pneumoniae  
Serratia marcescens  
Citrobacter freundii  
Proteus mirabilis  
Salmonella enteritidis  
Pseudomonas aeruginosa  
Haemophilus influenzae  
Haemophilus parainfluenzae  
Candida glabrata  
Candida albicans  
Candida krusei  
Candida parapsilosis  
Bacillus cereus  
Listeria monocytogenes  
Corynebacterium urealyticum  
Archaeobacterium haemolyticum  
Erysipelothrix rhusopathiae  
Stomatococcus  
Micrococcus luteus  
Staphylococcus saprophyticus  
Staphylococcus aureus  
Staphylococcus epidermidis  
Group A Streptococcus  
Streptococcus mitis  
Streptococcus pneumoniae  
Group B Streptococcus  
VanA Enterococcus faecium  
VanB Enterococcus faecium  
Non VanA/VanB Enterococcus faecium

Fig 3

L78253 Mouse Ly 49-H

atgagtgagc aggaggtcac tttcccaact atgagattcc acaagtcttc agggttgaac  
forward probe  
61 agccaggtga gacttgaggg gactcagaga tctagaaaag ctggcctaag agtccecttg  
reverse  
121 cagctcattg tgatagctct tggaatcctc tggtcccttc ggctggtaat tggtgcagt  
181 tttgtgacaa agttttttca gtatagtcaa cacaaacaag aaatcaatga aactctcaac  
241 caccgccata actgcagcaa catgcaaagg gatttcaact taaaggaaga aatggtgaca  
301 aataagtcta tagattgtag gccaaagctat gaacttctgg aatacatcaa aagagaacag  
361 gagagatggg acagtgaaac caagagtgtt tcagattctt cactgagacac aggcagaggt  
421 gttaaatact gggtctgcta tgggtactaaa tggtattatt tcatcatgaa taaaactaca  
481 tggagtggat gtaaagcgaa ctgccagcat tacagcgttc ccattgtgaa gatagaagat  
541 gaagatgaac tgaaattcct tcaacgccat gttattctag agagttactg gattggattg  
601 tcatatgata agaaaaaaaa ggaatgggca tggattcaca atggccaatc taaacttgac  
661 atgaaaataa agaaaatgaa ctttacgtct agaggatgtg tatttttatc taaagcaaga  
721 atagaagata ctgactgtaa tactccctac tactgtattt gtgggaagaa actggataaa  
781 ttcctgatt aa

Fig 4

## SEQUENCE LISTING

<110> University of Iowa Research Foundation

5        Dodgson, Kirsty Jane

<120> Method and Kit for Identifying Vancomycin-Resistant Enterococcus

10<130> 875.092WO1

<150> US 10/661,094

<151> 2003-09-12

15<160> 21

<170> FastSEQ for Windows Version 4.0

<210> 1

20<211> 1768

<212> DNA

<213> *Enterococcus faecium*

<400> 1

25gatatcggtta	cgcttcatgt	gccgctcaat	acggatacgc	actatattat	cagccacgaa	60
caaatacaga	gaatgaagca	aggagcattt	cttatcaata	ctgggcgcgg	tccacttgta	120
gatacctatg	agttgggttaa	agcattagaa	aacgggaaac	tgggcggtgc	cgcatggat	180
gtattggaag	gagaggaaga	gtttttctac	tctgattgca	cccaaaaacc	aattgataat	240
caatttttac	ttaaacttca	aagaatgcct	aacgtgataa	tcacaccgca	tacggcctat	300
30tataccgagc	aagcgttgcg	tgataccggt	gaaaaaacca	ttaaaaactg	tttggatttt	360
gaaaggagac	aggagcatga	atagaataaa	agttgcaata	ctgtttgggg	gttgctcaga	420
ggagcatgac	gtatcggtaa	aatctgcaat	agagatagcc	gctaacatta	ataaagaaaa	480
atacgagccg	ttatacattg	gaattacgaa	atctggtgta	tggaaaatgt	gcgaaaaacc	540
ttgcgcggaa	tgggaaaacg	acaattgcta	ttcagctgta	ctctcgccgg	ataaaaaaat	600
35gcacggatta	cttggttaaaa	agaaccatga	atatgaaatc	aaccatgttg	atgtagcatt	660
ttcagctttg	catggcaagt	caggtgaaga	tggatccata	caaggtctgt	ttgaattgtc	720
cggtatccct	tttgtaggct	gcgatattca	aagctcagca	atgtgtatgg	acaaatcggt	780
gacatacatc	gttgcgaaaa	atgctgggat	agctactccc	gccttttggg	ttattaataa	840
agatgatagg	ccggtggcag	ctacgtttac	ctatcctggt	tttgtttaagc	cggcgcgttc	900
40aggctcatcc	ttcggtgtga	aaaaagtcaa	tagcgcggac	gaattggact	acgcaattga	960
atcggcaaga	caatatgaca	gcaaaatctt	aattgagcag	gctgtttcgg	gctgtgaggt	1020
cggttgtgcg	gtattgggaa	acagtgccgc	gttagttggt	ggcgaggtgg	accaaatacag	1080
gctgcagtac	ggaatctttc	gtattcatca	ggaagtcgag	ccggaaaaag	gctctgaaaa	1140
cgcagttata	accgttcccg	cagacctttc	agcagaggag	cgaggacgga	tacaggaaac	1200

ggcaaaaaaa atatataaag cgctcggtcg tagaggtcta gcccggtggtg atatgttttt 1260  
 acaagataac ggcgcgattg tactgaacga agtcaatact ctgcccggtt tcacgtcata 1320  
 cagtcgttat ccccgatatga tggccgctgc aggtattgca cttcccgaac tgattgaccg 1380  
 cttgatcgta ttagcggttaa aggggtgata agcatggaaa taggatttac ttttttagat 1440  
 5gaaatagtag acgggtgttcg ttgggacgct aaatatgcca cttgggataa tttcaccgga 1500  
 aaaccgggtg acggttatga agtaaategc attgtaggga catacgagtt ggctgaatcg 1560  
 cttttgaagg caaaagaact ggctgctacc caagggtacg gattgcttct atgggacggg 1620  
 taccgtccta agcgtgctgt aaactgtttt atgcaatggg ctgcacagcc ggaaaataac 1680  
 ctgacaaagg aaagttatta tcccaatatt gaccgaactg agatgatttc aaaaggatac 1740  
 10gtggcttcaa aatcaagcca tagccgcg 1768

<210> 2

<211> 18

<212> DNA

15<213> *Enterococcus faecium*

<400> 2

ccgggtggcag ctacgttt

18

20<210> 3

<211> 27

<212> DNA

<213> *Enterococcus faecium*

25<400> 3

cctatcctgt ttttgtaag ccggcgc

27

<210> 4

<211> 20

30<212> DNA

<213> *Enterococcus faecium*

<400> 4

caccgaagga tgagcctgaa

20

35

<210> 5

<211> 1090

<212> DNA

<213> *Enterococcus faecalis*

40

<400> 5

tgctgcgaga taccacagaa .aacaatcagg aattgtctta actttgaaag gagtttacag

60

```

catgaataaa ,ataaaagtcg caattatctt cggcggttgc tcggaggaac atgatgtgtc 120
ggtaaaatcc gcaatagaaa ttgctgcgaa cattaatact gaaaaattcg atccgcacta 180
catcggaatt acaaaaaacg gcgtatggaa gctatgcaag aagccatgta cggaatggga 240
agccgatagt ctccccgcca tattctcccc ggataggaaa acgcatgggc tgcttgtcat 300
5gaaagaaaga gaatacgaac ctccgctgat tgacgtggct ttcccgggtt tgcatggcaa 360
atgcggggag gatggtgcga tacaggtctt gtttgaattg tctggtatcc cctatgtagg 420
ctgcgatatt caaagctccg cagcttgcgt ggacaaatca ctggcctaca ttcttacaaa 480
aaatgcgggc atcgcctgcc ccgaatttca aatgattgaa aaaggtgaca aaccggaggc 540
gaggacgctt acctaccctg tctttgtgaa gccggcacgg tcaggttcgt cctttggcgt 600
10aaccaaagta aacagtacgg aagaactaaa cgctgcgata gaagcagcag gacaatatga 660
tggaataatc ttaattgagc aagcgatttc gggctgtgag gtcggctgcg cggatcatggg 720
aaacgaggat gatttgattg tcggcgaagt ggatcaaatc cggttgagcc acggtatctt 780
ccgcatccat caggaaaaac agccggaaaa aggctcagag aatgcgatga ttatcgttcc 840
agcagacatt ccggtcgagg aacgaaatcg ggtgcaagaa acggcaaaga aagtatatcg 900
15ggtgcttgga tgcagagggc ttgctcgtgt tgatcttttt ttgcaggagg atggcggcat 960
cgttctaaac gaggtcaata ccctgcccgg ttttacatcg tacagccgct atccacgcat 1020
ggcggtgcc gcaggaatca cgcttccgcg actaattgac agcctgatta cattggcgat 1080
agagaggtga 1090

```

20&lt;210&gt; 6

&lt;211&gt; 18

&lt;212&gt; DNA

&lt;213&gt; Enterococcus faecalis

25&lt;400&gt; 6

cgacctcaca gcccgaaa

18

&lt;210&gt; 7

&lt;211&gt; 18

30&lt;212&gt; DNA

&lt;213&gt; Enterococcus faecalis

&lt;400&gt; 7

cgcttgctca attaagat

18

35

&lt;210&gt; 8

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Enterococcus faecalis

40

&lt;400&gt; 8

cggcaggaca atatgatgga a

21

&lt;210&gt; 9

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Enterococcus faecalis

5

&lt;400&gt; 9

cagcaggaca atatgatgga a

21

&lt;210&gt; 10

10&lt;211&gt; 801

&lt;212&gt; DNA

&lt;213&gt; Enterococcus faecium

&lt;400&gt; 10

15	aacgatgcg ccatacctcct gcaaaaaaag atcaacacgg gcaagccctc tgcataccaag	60
	cacccgatat actttctttg ccgtttcctg cacccgattt cgttcctcga ccggaatgtc	120
	tgcggaact gtaatcatcg cattttctga gcctttttcc ggctcgtttt cctgatggat	180
	gcggaagata ccgtggctca gccggatttg atccacttcg ccgacaatca aatcatcctc	240
	gttccccatg accgcacacc cgacctcaca gcccgaaatc gcttgctcaa ttaagatttt	300
20	tccatcatat tgcctgccc cttctatcgc agcgttaagt tcttcctgac cgtttacttt	360
	ggttacgcca aaggacgaac ctgaccgtgc cggcttcaca aagacagggg aggtaagcgc	420
	accgcctcc ggcttgctac ctttatcaat catttgaaat tcgggaacgg cgatgccgcg	480
	attttttgta agaattgtagg ccagtgttt gtccatgcaa gctgcggagc tttgaatata	540
	acagcccaca taggggatac cagacaatac aaacagcccc tgtatcgac catcctcccc	600
25	gcatttgcca tgcaaaaccg ggaaagccac atcaatacgc cgtgtttcgt attcgctttc	660
	tttcatgaca agcagcccat gcgttttcct atccggggag agtatggcgg ggagactgtc	720
	ggcttcccat tccgtacatg gcttcttgca tagcttccat acaccgtttt ttgtaattcc	780
	gatgtagtgc ggatcgaatt t	801

30&lt;210&gt; 11

&lt;211&gt; 801

&lt;212&gt; DNA

&lt;213&gt; Enterococcus faecium

35&lt;400&gt; 11

	aacgatgcg ccatacctcct gcaaaaaaag atcaacacga gcaagccctc tgcataccaag	60
	cacccgatat actttctttg ccgtttcctg cacccgattt cgttcctcga ccggaatgtc	120
	tgctggaacg ataatcatcg cattctctga gcctttttcc ggctcgtttt cctgatggat	180
	gcggaagata ccgtggctca accggatttg atccacttcg ccgacaatca aatcatcctc	240
40	gtttcccatg accgcgcagc cgacctcaca gcccgaaatc gcttgctcaa ttaagatttt	300
	tccatcatat tgcctgctg cttctatcgc agcgtttagt tcttcctgac tgtttacttt	360
	ggttacgcca aaggacgaac ctgaccgtgc cggcttcaca aagacagggg aggtaagcgt	420

```

cctcgctcc ggttgtcac ctttttcaat catttgaaat tcggggacgg cgatgcccgc 480
atrrtttgta agaagttagg ccagtgattt gtccatgcaa gctgcgagc tttgaatatc 540
gcagcctaca taggggatac cagacaattc aaacagaccc tgtatcgac catcctccc 600
gcatttgcca tgcaaaaccg ggaaagccac gtcaatacgc cgagtttcgt attctcttcc 660
5tttcatgaca agcagacccat gcgttttcct atccggggag aatatggcgg ggagactatc 720
ggcttcccat tccgtacatg gcttcttgca tagcttccat acgccgtttt ttgtaattcc 780
gatgtagtgc ggatcgaatt t 801

```

&lt;210&gt; 12

10&lt;211&gt; 801

&lt;212&gt; DNA

<213> *Enterococcus faecium*

&lt;400&gt; 12

```

15aacgatgccg ccacctcctt gcaaaaaaag atcaacacgg gcaagccctc tgcattcaag 60
caccgatgat actttctttg ccgtttcctg caccgatatt cgttcctcga ccggaatgtc 120
tgcggaact gtaatcatcg cattttctga gcctttttcc ggctcgtttt cctgatggat 180
gcggaagata ccgtggctca gccggatttg atccacttcg ccgacaatca aatcatcctc 240
gttccccatg accgcacacc cgacctcaca gcccgaaatc gcttgctcaa ttaagatttt 300
20tccatcatat tgtctgccg cttctatcgc agcgtaaagt tcttcctgac cgtttacttt 360
ggttacgcca aaggacgaac ctgaccgtgc cggcttcaca aagacagggt aggttaagcg 420
accgcctcc ggcttgcac cttttcaat aatttgaaat tcgggaacgg cgatgcccgc 480
atrrtttgta agaagttagg ccagtgattt gtccatgcaa gctgcgagc tttgaatatc 540
acagcccaca taggggatac cagacaatac aaacagcccc tgtatcgac catcctccc 600
25gcatttgcca tgcaaaaccg ggaaagccac atcaatacgc cgtgtttcgt attcgcttcc 660
tttcatgaca agcagcccat gcgttttcct atccggggag agtatggcgg ggagactgtc 720
ggcttcccat tccgtacatg gcttcttgca tagcttccat acaccgtttt ttgtaattcc 780
gatgtagtgc ggatcgaatt t 801

```

30&lt;210&gt; 13

&lt;211&gt; 801

&lt;212&gt; DNA

<213> *Enterococcus faecium*

35&lt;400&gt; 13

```

aacgatgccg ccacctcctt gcaaaaaaag atcaacacgg gcaagccctc tgcattcaag 60
caccgatgat actttctttg ccgtttcctg caccgatatt cgttcctcga ccggaatgtc 120
tgcggaact gtaatcatcg cattttctga gcctttttcc ggctcgtttt cctgatggat 180
gcggaagata ccgtggctca gccggatttg atccacttcg ccgacaatca aatcatcctc 240
40gttccccatg accgcacacc cgacctcaca gcccgaaatc gcttgctcaa ttaagatttt 300
tccatcatat tgtctgccg cttctatcgc agcgtaaagt tcttcctgac cgtttacttt 360
ggttacgcca aaggacgaac ctgaccgtgc cggcttcaca aagacagggt aggttaagcg 420

```

acccgcctcc	ggcttgtcac	ctttatcaat	aatttgaaat	tcggaacgg	cgatgccgc	480
atTTTTtgta	agaatgtagg	ccagtgattt	gtccatgcaa	gctgcggagc	tttgaatatc	540
acagcccaca	taggggatac	cagacaatac	aaacagcccc	tgtatcgac	catcctcccc	600
gcatttgcca	tgcaaaaccg	ggaaagccac	atcaatacgc	cgtgtttcgt	attcgctttc	660
5tttcatgaca	agcagcccat	gcgttttcc	atccggggag	agtatggcgg	ggagactgtc	720
ggcttcccat	tccgtacatg	gcttcttgca	tagcttccat	acaccgtttt	ttgtaattcc	780
gatgtagtgc	ggatcgaatt	t				801

&lt;210&gt; 14

10&lt;211&gt; 801

&lt;212&gt; DNA

<213> *Enterococcus faecium*

&lt;400&gt; 14

15aacgatgccg	ccatcctcct	gcaaaaaaag	atcaacacgg	gcaagccctc	tgcattcaag	60
cacccgatat	actttctttg	cgttttcctg	cacccgattt	cgttcctcga	ccggaatgtc	120
tgccgggaact	gtaatcatcg	cattttctga	gcctttttcc	ggctcgtttt	cctgatggat	180
gcggaagata	ccgtggctca	gccggatttg	atccacttcg	ccgacaatca	aatcatcctc	240
gttccccatg	accgcacacc	cgacctcaca	gcccgaatac	gcttgetcaa	ttaagatttt	300
20tccatcatat	tgctctgccg	cttctatcgc	agcgtaagt	tcttcctgac	cgtttacttt	360
ggttacgccca	aaggacgaac	ctgaccgtgc	cggcttcaca	aagacagggg	aggtaagcgc	420
acccgcctcc	ggcttgtcac	ctttatcaat	aatttgaaat	tcggaacgg	cgatgccgc	480
atTTTTtgta	agaatgtagg	ccagtgattt	gtccatgcaa	gctgcggagc	tttgaatatc	540
acagcccaca	taggggatac	cagacaatac	aaacagcccc	tgtatcgac	catcctcccc	600
25gcatttgcca	tgcaaaaccg	ggaaagccac	atcaatacgc	cgtgtttcgt	attcgctttc	660
tttcatgaca	agcagcccat	gcgttttcc	atccggggag	agtatggcgg	ggagactgtc	720
ggcttcccat	tccgtacatg	gcttcttgca	tagcttccat	acaccgtttt	ttgtaattcc	780
gatgtagtgc	ggatcgaatt	t				801

30&lt;210&gt; 15

&lt;211&gt; 630

&lt;212&gt; DNA

<213> *Enterococcus faecalis*

35&lt;400&gt; 15

gaggatgggt	gcattccagg	actgtttgaa	ttgtctggta	tcccctatgt	gggctgtgat	60
attcaaagct	ccgcagcttg	catggacaaa	tactggcct	acattcttac	aaaaaatgcg	120
ggcatcgccg	ttcccgaatt	tcaaatgatt	gataaagggt	acaagccgga	ggcgggtgcg	180
cttacctacc	ctgtctttgt	gaagccggca	cggtcagggt	cgtcctttgg	cgtaaccaa	240
40gtaaacggta	cggaagaact	taacgctgcg	atagaagcgg	caggacaata	tgatggaaaa	300
atcttaattg	agcaagcgat	ttcgggctgt	gaggtcgggt	gtgcggtcac	ggggaacgag	360
gatgatttga	ttgtcggcga	agtggatcaa	atccggctga	gccacgggat	cttccgcac	420

catcaggaaa acgagccgga aaaaggctca gaaaatgcga tgattacagt tcccgcagac 480  
attccggctcg aggaacgaaa tcgggtgcag gaaacggcaa agaaagtata tcgggtgctt 540  
ggatgcagag ggcttgcccg tgttgatctt tttttgcagg aggatggcgg catcgcttcta 600  
aatgaggtca acaccctgcc cggcttcacg 630

5  
<210> 16  
<211> 783  
<212> DNA  
<213> *Enterococcus faecalis*

10  
<400> 16  
gaaaaattcg atccgcacta catcgggaatt acaaaaagggt gtgtatggaa gctatgcaag 60  
aagccatgta cggaatggga agccgacagt ctecccgcca tactctcccc ggataggaaa 120  
acgcatgggtc tgcttgtcat gaaagaaagc gaatacgaaa cacggcgtat tgatgtggct 180  
15ttcccagttt tgcattggcaa atgcggggag gacgggtgcga tacagggttt atttgaattg 240  
tctggcatcc cctatgtggg ctgcgatatt caaagctccg cagcttgcat ggacaaatca 300  
ctggcctaca ttcttataaa aaatgcgggc atcgccgttc ccgaatttca aatgattgat 360  
aaaggtgaca agccggagac ggggtgcgctt acctaccctg tctttgtgaa gccggcacgg 420  
tcagggttcgt cctttggctt aaccaaagta aacggtagcg aagaacttaa cgctgcgata 480  
20gaagcggcag gacaatatga tggaaaaatc ttaattgagc aagcgatttc gggctgtgag 540  
gtcggctgtg cggttatggg gaacgaggat gatttgattg tcggcgaaat ggatcaaatc 600  
cggctgagcc atggtatctt ccgcatccat caggaaaacg agccggaaaa aggatcagag 660  
aatgcgatga ttaccgttcc tgcagacatc ccagtcgggg aacgaaatcg ggtgcaggaa 720  
acggcaaaga aagtatatcg ggcgcttggg tgcagagggc ttgcccggtg tgatcttttt 780  
25ttg 783

<210> 17  
<211> 801  
<212> DNA  
30<213> Artificial Sequence

<220>  
<223> A synthetic consensus sequence

35<400> 17  
aacgatgccg ccatectcct gcaaaaaaag atcaacacgg gcaagccctc tgcattcaag 60  
caccgatgat actttctttg ccgtttcctg caccgatatt cgttcctcga ccggaatgtc 120  
tgccggaaact gtaatcatcg cattttctga gcctttttcc ggctcgtttt cctgatggat 180  
gcggaagata ccgtgggtca gccggatttg atccacttcg ccgacaatca aatcatcctc 240  
40gttccccatg accgcacacc cgacctcaca gcccgaaatc gcttgctcaa ttaagatttt 300  
tccatcatat tgcctcgccg ctctctatcg agcgttaagt tcttccgtac cgtttacttt 360  
ggttacgcca aaggacgaac ctgacctgac cggcttcaca aagacagggt aggttaagcgc 420

8

acccgcctcc ggcttgtcac ctttatcaat catttgaaat tcgggaacgg cgatgcccgc	480
atTTTTtTgta agaattgtagg ccagtgattt gtccatgcaa gctgCGgagc tttgaatata	540
acagcccaca taggggatac cagacaattc aaacagcccc tgtatcgac catcctcccc	600
gcatttgcca tgcaaaaccg ggaaagccac atcaatacgc cgtgtttcgt attcgctttc	660
5tttcatgaca agcagcccat gcgttttccat atccggggag agtatggcgg ggagactgtc	720
ggcttcccat tccgtacatg gcttcttgca tagcttccat acaccgtttt ttgtaattcc	780
gatgtagtgc ggategaatt t	801

&lt;210&gt; 18

10&lt;211&gt; 792

&lt;212&gt; DNA

&lt;213&gt; Mus musculus

&lt;400&gt; 18

15atgagtgagc aggaggtcac tttcccaact atgagattcc acaagtcttc aggggtgaac	60
agccaggtga gacttgagg gactcagaga tctagaaaag ctggcctaag agtcccttgg	120
cagctcattg tgatagctct tggaatcctc tgttcccttc ggctggtaat tgttgcaagt	180
tttTtgacaa agttttttca gtatagtcaa cacaaacaag aaatcaatga aactctcaac	240
caccgccata actgcagcaa catgcaaagg gatttcaact taaaggaaga aatgttgaca	300
20aataagtcta tagattgtag gccaagctat gaacttctgg aatacatcaa aagagaacag	360
gagagatggg acagtgaaac caagagtgtt tcagattctt cacgagacac aggcagaggt	420
gttaaatact ggttctgcta tggactaaa tgttattatt tcatcatgaa taaaactaca	480
tggagtggat gtaaagcgaa ctgccagcat tacagcgttc ccattgtgaa gatagaagat	540
gaagatgaac tgaaattcct tcaacgccat gttattctag agagttagtg gattggattg	600
25tcatatgata agaaaaaaaa ggaatgggca tggattcaca atggccaatc taaacttgac	660
atgaaaataa agaaaatgaa ctttacgtct agaggatgtg tatttttata taaagcaaga	720
atagaagata ctgactgtaa tactccctac tactgtattt gtgggaagaa actggataaa	780
ttccctgatt aa	792

30&lt;210&gt; 19

&lt;211&gt; 23

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

35&lt;220&gt;

&lt;223&gt; A synthetic primer

&lt;400&gt; 19

gctggcctaa gagtgtgttc agt

23

40

9

&lt;210&gt; 20

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Mus musculus

5

&lt;400&gt; 20

agccgaaggg aacagaggat

20

&lt;210&gt; 21

10&lt;211&gt; 29

&lt;212&gt; DNA

&lt;213&gt; Mus musculus

&lt;400&gt; 21

15ccttggcagc tcattgtgat agctccttgg

29

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
31 March 2005 (31.03.2005)

PCT

(10) International Publication Number  
**WO 2005/028679 A3**

(51) International Patent Classification<sup>7</sup>: C12Q 1/68

(21) International Application Number:  
PCT/US2004/029602

(22) International Filing Date:  
13 September 2004 (13.09.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
10/661,094 12 September 2003 (12.09.2003) US

(71) Applicant (for all designated States except US): UNIVER-  
SITY OF IOWA RESEARCH FOUNDATION [US/US];  
Technology Innovation Center, Suite #214, 100 Oakdale  
Campus, Iowa City, IA 52242 (US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): DODGSON, Kirsty,  
Jane [GB/US]; 124 Grove Street, Iowa City, IA 52246  
(US).

(74) Agents: STEFFEY, Charles, E. et al.; Schwegman, Lund-  
berg, Woessner & Kluth, P.O. Box 2938, Minneapolis, MN  
55402 (US).

(81) Designated States (unless otherwise indicated, for every  
kind of national protection available): AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,  
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,  
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,  
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,  
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,  
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,  
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,  
ZW.

(84) Designated States (unless otherwise indicated, for every  
kind of regional protection available): ARIPO (BW, GH,  
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,  
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,  
FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI,  
SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,  
GW, ML, MR, NE, SN, TD, TG).

**Published:**

- with international search report
- before the expiration of the time limit for amending the  
claims and to be republished in the event of receipt of  
amendments

(88) Date of publication of the international search report:  
16 June 2005

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: METHOD AND KIT FOR IDENTIFYING VANCOMYCIN-RESISTANT ENTEROCOCCUS

(57) Abstract: The invention provides a method to process samples for DNA detection and a method to identify the vancomycin  
resistance gene status of an organism.

WO 2005/028679 A3

## INTERNATIONAL SEARCH REPORT

Inter: onal Application No  
PCT/US2004/029602

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data, Sequence Search

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>PATEL R. ET AL.,: "multiplex pcr detection of vanA,vanB,vanC-1, and van C2/3 genes in Enterococci" J. CLIN. MICROBIOLOGY, vol. 35, no. 3, March 1997 (1997-03), pages 703-707, XP002321621 the whole document</p> <p style="text-align: center;">-/-</p>	1-47

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier document but published on or after the international filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*8\* document member of the same patent family

Date of the actual completion of the international search

18 March 2005

Date of mailing of the international search report

19/04/2005

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Mueller, F

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US2004/029602

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>PETRICH A K ET AL: "Direct detection of vanA and vanB genes in clinical specimens for rapid identification of vancomycin resistant enterococci (VRE) using multiplex PCR"</p> <p>MOLECULAR AND CELLULAR PROBES, ACADEMIC PRESS, LONDON, GB, vol. 13, no. 4, August 1999 (1999-08), pages 275-281, XP004441556 ISSN: 0890-8508 cited in the application see whole doc. esp. p.277, 1. col., 2. par. ff.</p>	1-47
X	<p>SATAKE S. ET AL.,: "detection of vancomycin-resistant enterococci in fecal samples by PCR"</p> <p>J. CLIN. MICROBIOLOGY, vol. 35, no. 9, September 1997 (1997-09), pages 2325-2330, XP002321622 the whole document</p>	1-47
X	<p>SAHM D. S. ET AL.,: "rapid characterization schemes for surveillance isolates of vancomycin-resistant enterococci"</p> <p>J. CLIN. MICROBIOLOGY, vol. 35, no. 8, August 1997 (1997-08), pages 2026-2030, XP002321623 cited in the application the whole document</p>	1-47
X	<p>WO 99/01571 A (ID BIOMEDICAL CORPORATION; MODRUSAN, ZORA, D) 14 January 1999 (1999-01-14) see whole doc. esp. claims, seq id 17 is partial homologue to claimed seq id 6</p>	1-47
X	<p>US 6 001 564 A (BERGERON ET AL) 14 December 1999 (1999-12-14) see whole doc. esp. claims</p>	1-47
X	<p>WO 01/23604 A (INFECTIO DIAGNOSTIC INC; BERGERON, MICHEL, G; BOISSINOT, MAURICE; HUL) 5 April 2001 (2001-04-05) see whole doc. esp. claim 21</p>	1-47

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US2004/029602

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9901571	A	14-01-1999	AU 8327398 A	25-01-1999
			CA 2294565 A1	14-01-1999
			WO 9901571 A2	14-01-1999
			EP 0996743 A2	03-05-2000
			JP 2002507129 T	05-03-2002
			US 6274316 B1	14-08-2001
US 6001564	A	14-12-1999	US 2003180733 A1	25-09-2003
			US 2002055101 A1	09-05-2002
			US 5994066 A	30-11-1999
			AT 219524 T	15-07-2002
			AU 705198 B2	20-05-1999
			AU 3468195 A	29-03-1996
			BR 9508918 A	21-10-1997
			CA 2199144 A1	21-03-1996
			WO 9608582 A2	21-03-1996
			DE 69527154 D1	25-07-2002
			DE 69527154 T2	16-01-2003
			DK 804616 T3	07-10-2002
			EP 1138786 A2	04-10-2001
			EP 0804616 A2	05-11-1997
			ES 2176336 T3	01-12-2002
			JP 10504973 T	19-05-1998
			NO 971111 A	09-05-1997
			NZ 292494 A	25-03-1998
			PT 804616 T	29-11-2002
WO 0123604	A	05-04-2001	CA 2283458 A1	28-03-2001
			CA 2307010 A1	19-11-2001
			AU 7636000 A	30-04-2001
			BR 0014370 A	05-11-2002
			WO 0123604 A2	05-04-2001
			CA 2388461 A1	05-04-2001
			EP 1246935 A2	09-10-2002
			JP 2003511015 T	25-03-2003